EXPOSURE TO ZOONOTIC *STAPHYLOCOCCUS AUREUS* AMONG INDUSTRIAL HOG OPERATION WORKERS AND THEIR HOUSEHOLD CONTACTS IN NORTH CAROLINA, AND DISSEMINATION INTO THE HOUSEHOLD ENVIRONMENT

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ABSTRACT

Maya Nadimpalli: Exposure to zoonotic *Staphylococcus aureus* among industrial hog operation workers and their household contacts in North Carolina, and dissemination into the household environment
(Under the direction of Jill Stewart)

Industrialized systems of food animal production are a potential source of exposure to antibiotic-resistant *Staphylococcus aureus* that can be transmitted between animals and humans. In the United States, there is little information regarding occurrence and persistence of exposure to *S. aureus* among healthy individuals who have frequent contact with intensively-raised livestock, or about occupational activities that may be associated with exposure, health implications of exposure, or dissemination of these bacteria into the household environment. This dissertation sought to address these research gaps. In Chapter 2, I describe findings from a 14-day, repeated measures pilot study in which we observed persistent nasal carriage with zoonotic *S. aureus* among industrial hog operation workers, even during time away from work. In Chapters 3 and 4, I describe findings from a four month repeated-measures study of workers and their household members, in which we observed that (1) infrequent face mask use was a predictor of workers' nasal carriage with zoonotic *S. aureus*, (2) presence of zoonotic *S. aureus* in workers' noses may be associated with recently reported symptoms of skin and soft tissue infection, (3) workers in North Carolina frequently carry a *S. aureus* strain type commonly detected in Asia (CC9), and (4) zoonotic *S. aureus* may be shared between workers and their household members. In Chapter 5, I describe findings from a household environmental sampling study in which we observed that households' environmental exposure to industrial hog operations was associated with presence of zoonotic *S. aureus* in the home, and that *S. aureus* in household members' noses was similar to what we recovered from their household

environment. Overall, the findings outlined in this dissertation suggest that current livestock production practices can lead to persistent nasal carriage of zoonotic *S. aureus* among workers as well as their household members, and that some of these antibiotic-resistant strains can have reservoirs in the household environment. Additional research is necessary to determine public health risks associated with these zoonotic, antibiotic-resistant bacteria. These research findings could be used to help inform national policies about food animal production practices such that worker and community health may be safeguarded.

To Pavan, and to my loving family, who have always encouraged me
to be the best person I can.

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LIST OF ABBREVIATIONS

CAFO	Confined animal feeding operation
CC	Clonal complex
CFU	Colony forming unit
CoNS	Coagulase-negative staphylococci
CSA	CHROMagar™ Staph aureus
MDRSA	Multidrug-resistant *Staphylococcus aureus*
MLST	Multilocus sequence typing
MLVA	Multilocus variable number tandem repeat analysis
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSSA	Methicillin-susceptible *Staphylococcus aureus*
PBP	Penicillin binding protein
PCR	Polymerase chain reaction
PFGE	Pulse-field gel electrophoresis
PPE	Personal protective equipment
REACH	Rural Empowerment for Community Help
SCC*mec*	Staphylococcal cassette chromosome *mec*
ST	Sequence type
WGST	Whole genome sequence typing

CHAPTER ONE: INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION

Industrialized systems of food animal production are a potential source of exposure to zoonotic bacteria, including bacteria that can be transmitted between animals and humans. Healthy individuals who have frequent contact with industrially-produced livestock, such as industrial hog workers, may be at an increased risk of exposure to zoonotic bacteria and may serve as a bridging population for cross-species exposure, adaptation, and transmission to the community. The bacterium *Staphylococcus aureus* is a pathogen of clinical and public health significance to which livestock workers may be exposed through their work activities.

Despite recognition of the potential health risks associated with exposure to *S. aureus* (Tara C. Smith & Pearson, 2011), there is limited surveillance of livestock workers and their household contacts in regions where industrial livestock production is particularly concentrated in the United States, such as in eastern North Carolina. Thus, there is little information regarding occurrence and persistence of exposure to *S. aureus* among this population, or about occupational activities that may be associated with exposure, health implications of exposure, or dissemination of these bacteria into the household environment.

This dissertation describes findings from targeted, longitudinal surveillance studies of *S. aureus* exposure among industrial livestock workers and their household contacts in eastern North Carolina. Since, to our knowledge, longitudinal surveillance studies of *S. aureus* exposure had never before been conducted in North Carolina, we first conducted a 14-day, repeated measures pilot study among a small cohort of livestock workers to examine whether there were associations between short times away from work and nasal carriage of zoonotic *S. aureus*. We used findings from this pilot study to inform the design of a four-month, repeated-measures study of nasal carriage of zoonotic *S. aureus* among a larger cohort of livestock workers as well as their household contacts. Finally, we conducted a cross-sectional household sampling study nested within this four-month study in order to provide insight into the role of the household environment as a reservoir for zoonotic bacteria exposure.

The work was possible by leveraging existing collaborations between investigators at UNC-Chapel Hill and the Rural Empowerment Association for Community Help (REACH), a community-based organization located in eastern North Carolina. Through ongoing community outreach efforts, REACH has earned the trust of industrial livestock workers and their household members, a population that would otherwise be prohibitively difficult for researchers to engage for surveillance studies. This work is timely and important given the limited understanding of the burden of exposure to zoonotic *S. aureus* among industrial livestock workers and their household members in the United States. Our results provide novel information about the associations between exposure to the industrial livestock production environment, nasal carriage of zoonotic *S. aureus*, and dissemination of zoonotic *S. aureus* into the household environment. These findings could be used to improve zoonotic pathogen transmission prevention efforts, and to inform national policies about food animal production practices such that worker and community health may be safeguarded.

RESEARCH OBJECTIVES AND RATIONALE

The overall purpose of this research was to improve the knowledge base regarding occurrence and persistence of exposure to antibiotic-resistant *S. aureus* among livestock workers and their household members in the United States. Specifically, we aimed to improve our understanding of occupational activities that may be associated with exposure, health implications of exposure, and dissemination of these bacteria into the household environment. These goals were achieved through four main research objectives.

Objective 1. To examine the occurrence and persistence of livestock-associated *S. aureus* nasal carriage among up to 25 workers at industrial hog production operations before, during, and after at least 24 hours away from work by:

a. Genetically and phenotypically characterizing *S. aureus* detected on nasal swabs during a 14-day study period,

b. Assessing whether time away from work is associated with changes in livestock-associated *S. aureus* nasal carriage during this 14-day study period,

c. Assessing whether fixed personal and/or occupational exposures are associated with persistence of nasal carriage,

d. Assessing whether livestock-associated *S. aureus* nasal carriage is associated with symptoms of infection, if such symptoms are reported.

Rationale

Previous work has demonstrated that industrial livestock workers and household members in the United States are exposed to livestock-associated *S. aureus*, including livestock-associated methicillin-resistant (MRSA) and multidrug-resistant *S. aureus* (MDRSA) (Rinsky et al., 2013; Smith & Pearson, 2011). However, persistence of livestock-associated *S. aureus* in the nasal passages of this population has not been investigated. Strains of human-associated *S. aureus* can persist in the nose for months to years, and persistence has been linked to an increased risk of infection in clinical settings (Wertheim et al., 2005). Understanding the ability of livestock-associated, antibiotic-resistant *S. aureus* to persistently colonize the human nose, particularly during time away from work, is therefore essential in determining the magnitude and severity of the public health risk posed by transmission of these bacteria.

Because this research question has never been investigated in the United States, we determined that a pilot study was the most practical way to assess the potential for losses in nasal colonization following short times away from work, before expanding to a larger study.

Objective 2. To examine baseline occurrence of livestock-associated *S. aureus* nasal carriage among up to 200 industrial hog operation workers and their household contacts by:

a. Examining the baseline prevalence and distribution of *S. aureus*-related outcomes, including *S. aureus* with indicators of livestock-association;

b. Assessing whether certain personal and/or occupational exposures are associated with binary and quantitative baseline *S. aureus* nasal carriage,

c. Assessing whether baseline binary and quantitative *S. aureus* nasal carriage is associated with symptoms of recent skin and soft tissue infection among workers and their household contacts, if such symptoms are reported.

Rationale

We designed a four month, repeated-measures study to assess *S. aureus* nasal carriage among industrial livestock operation workers and household members in response to findings from the 14-day repeated measures study of 22 industrial hog operation workers in North Carolina that we conducted in 2012. In the 14-day study, we observed that 45.5% of participants persistently carried livestock-associated *S. aureus* during the follow-up period, despite time away from work. However, findings from the 14-day study have limitations. Because of small sample size and because we observed few changes in *S. aureus* presence/absence during the 14-day follow-up period, we were unable to draw conclusions about potential statistical associations between *S. aureus* nasal carriage and exposures that vary over time (e.g. time away from work) or exposures that remain fixed over time (e.g. personal characteristics). Thus, our results suggested that a study among a larger cohort of workers, for whom *S. aureus* nasal carriage is quantified rather than evaluated as present vs. absent, may be necessary to evaluate measures of association between nasal carriage and fixed personal/occupational exposures.

In order to introduce the cohort which participated in the four month longitudinal study and to present our novel evaluation of *S. aureus* nasal carriage among hog operation workers as a continuous rather than binary outcome, we first chose to evaluate these research questions in the context of the baseline data alone.

Objective 3. To examine persistence of livestock-associated *S. aureus* nasal carriage among up to 200 industrial hog operation workers and their household contacts sampled bi-weekly over four months by:

a. Examining the prevalence and distribution of *S. aureus*-related nasal carriage states (e.g. persistent, intermittent, non-carrier) among workers and their household contacts;
b. Assessing differences in bacterial loads between non-carriers, intermittent carriers, and persistent carriers,
c. Assessing whether typical personal and/or occupational exposures are associated with persistence of nasal carriage,
d. Evaluating whether average within-worker changes in personal and occupational exposures the week prior to nasal sampling are associated with average within-worker changes in nasal carriage of *S. aureus* and related outcomes over four months;
e. Assessing whether persistent *S. aureus* nasal carriage is associated with symptoms of skin and soft tissue infection among workers and their household contacts, if such symptoms are reported.

Rationale

At the time of this study's inception, the temporal dynamics of *S. aureus* nasal carriage had never before been evaluated among a livestock-exposed cohort of this size in the United States. Four months of follow-up was determined to be sufficient time to capture major changes in occupational exposures (e.g. herd replacements at the hog operation, switching employment to a different hog operation, or ending employment at the hog operation) that could affect nasal carriage of livestock-associated *S. aureus*. We hypothesized that the higher power afforded by increased sample size, a repeated-measures study design, and evaluation of *S. aureus* as a continuous outcome would improve our ability to evaluate measures of association between nasal carriage and fixed personal/occupational exposures.

Objective 4. To investigate the household environment as a potential reservoir of antibiotic-resistant *S. aureus* with indicators of livestock-association by:

a. Genetically and phenotypically characterizing *S. aureus* detected on surface samples from a subset of up to 30 households participating in the four-month longitudinal study;
b. Evaluating whether households' relative geographic exposure to these operations is associated with the percent of household environmental surfaces contaminated with *S. aureus* and related outcomes; and
c. Examining concordance between *S. aureus spa* types recovered from the household environment and *S. aureus spa* types recovered from the noses of household members ≤ two weeks prior to household environmental sampling.

Rationale

Although previous work has shown that *S. aureus* can persist in the household environment (Davis, Iverson, et al., 2012; Masterton et al., 1995), to the best of our knowledge, no study has investigated the contribution of the household environment to nasal carriage of antibiotic-resistant *S. aureus* among livestock workers and their household members.

Investigation of the household environment as a potential reservoir is particularly critical among livestock workers in North Carolina, given pilot data suggesting persistence of nasal carriage occurs even during time away from work (collected for Aim 1), and the density of industrial food animal production facilities (hog, turkey, poultry) in the region where participants in this study lived and worked. Contamination of the household environment could play an important and as of yet uninvestigated role in the transmission dynamics of livestock-associated *S. aureus*.

LITERATURE REVIEW

Industrialized systems of food animal production are a potential source of exposure to bacterial pathogens, including those that can be transmitted between animals and humans (Chomel et al., 2007). Healthy individuals who have frequent contact with industrially-produced livestock, such as industrial hog and poultry workers, may be at an increased risk of exposure to zoonotic pathogens and may serve as a bridging population for cross-species exposure, adaptation, and transmission to their households and to the broader community. The bacterium *Staphylococcus aureus* is a pathogen of clinical and public health significance to which livestock workers may be exposed to through their work activities.

Industrial food animal production in the United States

Within the past sixty years, animal production in the United States has shifted from many small, diversified family farms to fewer, larger, and highly specialized factory farms. Today, confined animal feeding operations (CAFOs) produce the majority of food animals raised in the US, and a small number of vertically-integrated hog and poultry corporations oversee all aspects of the food animal production process. Vertically integrated food animal production corporations are characterized by standardization of feed, selective in-breeding of animals, non-therapeutic dosing of animals with antibiotics to promote growth, and mechanization of feeding, watering, and other husbandry activities (Pew Charitable Trusts, 2008). The number of animals that are grown on animal production operations has surged – between 1994 and 2008, the number of animals per hog operation increased by 180%, while the number of chickens per broiler operation increased by 130% (Pew Charitable Trusts, 2008). By raising more animals within a confined space, hog and poultry corporations are able to create “economies of scale,” shorten the time between birth and slaughter, increase their profit margins, and respond to the growing global demand for meat.

Changes in the production process have resulted in changes in the size and composition of the labor force on food animal production operations, as well. As family farms have converted to or been replaced by large, industrial-scale operations, the percent of operations relying on hired farm workers has increased (Martin & Taylor, 2013). However, increases in hired labor have been vastly outpaced by increases in animal inventory. Therefore, despite a higher number of average employees per farm, workers at industrial food animal production operations in the United States are exposed to a greater number of food animals per capita than ever before.

Antibiotic use in industrial food animal production

Twenty million pounds of antibiotics, or 75% of all antibiotics sold in the United States, are sold each year for use in industrially-raised food animals (Food and Drug Administration, 2011). Antibiotics are used in industrial food animal production for three main purposes: (1) therapeutically, to treat animals exhibiting signs of illness or infection; (2) prophylactically, to prevent or protect against disease; and (3) for growth promotion, by modifying animals’ gut microflora to increase feed efficiency (Mcewen & Fedorka-Cray, 2002). The Pew Charitable Trusts has estimated that approximately 85% of the antibiotics given to food animals each year are for these latter two purposes (Pew Charitable Trusts, 2008). Tetracycline, one the most heavily used antibiotics in food animal production in the United States (Love et al., 2011), is primarily administered for growth promotion. However, it is estimated that more than half of the classes of antibiotics licensed for use in pigs are also used in human medicine (Mellon et al., 2001), and approximately 75% of the estimated 24.6 million pounds of antibiotics consumed for nontherapeutic purposes by livestock each year is excreted (Chee-Sanford et al., 2009). Industrial food animal production has thus been linked to an increased animal and environmental reservoir of novel antibiotic-resistant bacterial pathogens that may cause disease in humans (Aarestrup, 1995; Mathew et al., 1999).

Industrial food animal production in North Carolina

Industrial food animal production has grown rapidly in North Carolina in the past few decades, with the state moving from fifteenth (in 1982) to second (in 2007) in hog production nationally, and from fourth to third in poultry production. As of 2007, an estimated 10 million hogs are grown annually on approximately 2,500 hog operations in North Carolina (Census of Agriculture, 2007; Wing et al., 2000). The number of individuals working on hog production operations in North Carolina is difficult to determine, but census data suggest that approximately 6,000 workers are employed at the 938 hog operations that report hired labor (Census of Agriculture, 2007). North Carolina's hog CAFOs are concentrated in the southern Piedmont and in the eastern coastal plain of the state, with the highest densities occurring in Duplin and Sampson counties. Together, hog production in these two counties accounts for nearly half of the state's total production. The hog-to-resident ratio in Duplin County is reported to be one of the highest in the world. Growth in the hog industry has leveled off in the past several years due to a 1997 moratorium on permitting new hog CAFO liquid waste storage lagoons (North Carolina Department of Agriculture and Consumer Services, 2010). Meanwhile, poultry production in North Carolina has increased in recent years. Turkey production is highest in Duplin and Sampson counties, but egg production occurs in the central part of the state and broiler production is increasing throughout the state (Census of Agriculture, 2007). As of 2007, an estimated 800 million layer chickens, pullets, turkeys, broilers and other meat-type chickens are grown annually on approximately 4,000 farms (Census of Agriculture, 2007). Approximately 9,000 workers are employed at the 1,511 of the approximately 4,000 poultry and egg production operations that report hired labor (Census of Agriculture, 2007).

Staphylococcus aureus

Staphylococci are ubiquitous, gram-positive cocci that tend to cluster in chains and are capable of surviving under both aerobic and anaerobic conditions. Staphylococci can asymptomatically colonize the skin, skin glands, and mucous membranes of warm-blooded animals (Crossley & Archer, 1997), but are also opportunistic pathogens that can cause invasive infections. Of at least 32 species of staphylococci, *Staphylococcus aureus* is the most pathogenic among humans.

The structure of *S. aureus* and its production of virulence factors and exotoxins are critically related to its fitness as a human pathogen. Like all staphylococci, *S. aureus* have a thick cell wall composed of peptidoglycan, and several serotypes also have a protective polysaccharide capsule that facilitates adherence to surfaces and evasion of the immune system (Murray et al., 2002). Several strains of *S. aureus* are capable of producing a variety of exotoxins, including TSST-1 (associated with toxic shock syndrome), exfoliative toxins (associated with staphylococcal scaled skin syndrome), staphylococcal enterotoxin B (associated with food poisoning), and α toxin, β toxin, and Panton-Valentine leukocidin (all associated with necrotizing pneumonia) (Lowy, 1998). The production of these toxins allows *S. aureus* to cause a wide range of invasive and potentially lethal infections.

Antibiotic resistance

S. aureus has developed a resistance mechanism for the majority of classes of antibiotics produced by humans, including many last-line-of-defense antibiotics (Skov, 2011). There are several mobile genetic elements (MGEs) that encode resistance mechanisms in *S. aureus*, although the best-studied is the Staphylococcal Cassette Chromosome *mec* (SCC*mec*) element, which carries the *mecA* gene. Expression of *mecA* confers resistance to all β-lactam antibiotics, including carbanepems and cephalosporins; *mecA*-positive *S. aureus* are referred to as MRSA. In addition to the *mecA* gene, certain SCC*mec* complexes (namely types II and III) encode additional drug resistance genes on integrated mobile elements (Skov, 2011).

S. aureus can carry resistance genes inserted at other sites on the chromosome, such as on transposons or phages, as well as on circular plasmids (Deurenberg et al., 2007). Resistance genes carried by transposons, phages, and plasmids can encode proteins that disrupt the normal function of antibiotics through a variety of mechanisms, including active efflux of the antibiotic from the cell, elimination or reduction of antibiotic binding to the cell target, enzymatic cleavage or modification of the antibiotic in order to inactivate it, metabolic bypass by the cell of the pathway inhibited by the antibiotic, and overproduction of the antibiotic's target, among other mechanisms (Mayers et al., 2009). Additionally, *S. aureus* can develop resistance through random mutation of existing genes. Such mutations require selective pressure to maintain themselves in subsequent generations.

Epidemiology of *S. aureus* carriage in humans and animals

<u>Humans</u>

Though *S. aureus* can be highly pathogenic, only a fraction of the *S. aureus* that occurs among humans actually causes disease. According to data from the 2001-2004 National Health and Nutrition Examination Survey (NHANES), approximately 1/3 of American men and women harbor *S. aureus* in their nasal passages (Gorwitz et al., 2008), which is the preferential site for *S. aureus* colonization. Approximatley 50% of the population carry it on their skin, hair, or in their throats (Bhatia & Zahoor, 2007). Nasal carriage of *S. aureus* can be transient. Longitudinal studies suggest that only 20-30% of the population are persistent, "true" carriers of *S. aureus*, while the remaining portion of the population are either intermittently colonized or not colonized at all (Van Belkum et al., 2009). Though concrete population determinants of persistent carriage remain speculative, race, genetics, immune characteristics, strain diversity, and environment may play a role. While nasal carriage is a risk factor for infection in the clinical setting, most individuals who are colonized never become infected (Gorwitz et al., 2008). Eradication of nasal colonization via mupirocin treatment may or may not prevent subsequent *S. aureus* infection (Wertheim et al., 2005).

Nasal carriage of methicillin-resistant strains of *S. aureus* is far less common than nasal carriage of *S. aureus* in general. As of 2005, MRSA was estimated to be present in the noses of 1.5% of the US population, though rates were steadily increasing (Gorwitz et al., 2008). Risk factors for MRSA acquisition include hospitalization, use of antibiotics, previous colonization with MRSA, drug use, old age, low socioeconomic status (Charlebois et al., 2002; Gorwitz et al., 2008), imprisonment (Aiello et al., 2006), use of work-out gyms (Kirkland & Adams, 2008), and pig farming (Voss et al., 2005). While hospital-acquired MRSA strains were once predominant in the US, they are largely being replaced by community-acquired strains, particularly in the hospital setting.

<u>Animals</u>

The epidemiology of *S. aureus* carriage among animals is poorly understood. *S. aureus* is thought to be a commensal organism of at least pigs and cows, and a transient colonizer of many other domesticated mammals. Among pigs, cross-sectional studies of *S. aureus* nasal carriage have revealed prevalence estimates between 1-39% (De Neeling et al., 2007; Neela et al., 2009). *S. aureus* infections among pigs, such as pyemia (microabscesses), are uncommon; the prevalence of pyemia among pigs in Denmark is 0.4% (Nielsen et al., 2009). Among cattle, cross-sectional studies of *S. aureus* nasal carriage have revealed prevalence estimates between 0%-28% (Graveland et al., 2010; Weese et al., 2012). Bovine mastitis, often caused by *S. aureus*, is a common infection among cattle.

Only a handful of studies have examined the epidemiology of nasal carriage of *S. aureus* among household pets, specifically cats and dogs. Among cats, *S. aureus* is thought to be a commensal capable of causing endogenous infections; 44% of cats were colonized with *S. aureus* in one cross-sectional study in Japan (Sasaki et al., 2012). Among dogs, *S. aureus* is not thought to be a commensal organism (Weese & Van Duijkeren, 2010). However, there is some evidence suggesting that nasal carriage may occur when humans in the same household are colonized; in these situations, dogs may act as an additional household reservoir for *S.*

aureus and MRSA (Baptiste et al., 2005). Humans who work in healthcare settings are more likely to transmit *S. aureus*, including MRSA, to dogs in their households (Boost et al., 2008).

***S. aureus* survival in the environment**

Though warm-blooded animals are its primary reservoir, *S. aureus* has also been detected in air (Bassetti et al., 2005), in drinking and waste waters (Lechevallier & Seidler, 1980; Rosenberg-Goldstein, 2010), and in food (Loir et al., 2003). It has been demonstrated that *S. aureus* can persist in river and sea water for up to two weeks (Tolba et al., 2008), as well as on dry surfaces for days to months (Kramer et al., 2006). *S. aureus* has also been detected in hog and poultry waste (Dimitracopoulos et al., 1977; Graham et al., 2009). Humans can transmit *S. aureus* to their surrounding environment through direct contact, shedding of skin cells, aerosol discharge (e.g. sneezing) , and/or gastrointestinal routes (Davis, Iverson, et al., 2012). Simultaneously, *S. aureus* in the environment can be transferred to the nose or other colonization sites via the hands (Wertheim et al., 2005). Contamination of hospital and household environments with *S. aureus* has been linked to recurrent colonization and infection among susceptible populations (Dancer, 2009; Fritz et al., 2014).

The environmental hardiness of *S. aureus* is likely related to its ability to withstand desiccation for extended periods of time. *Staphylococci* are unusually resistant to desiccation (Clements & Foster, 1999). Desiccation tolerance among *S. aureus* is believed to be regulated by the production of three specific proteins: *clp*X, *sig*B and *yjb*H, which are involved in protein turnover and stress response. Temperature, humidity, the stage of *S. aureus* growth at the time of exposure to dry conditions (lag phase, log phase, post-exponential growth), and the density of *S. aureus* cell contamination affect *S. aureus* survival in the environment (Chaibenjawong & Foster, 2011). Depending on these conditions, culturable *S. aureus* may survive in the environment for as little as a few hours to at least 1,097 days (Chaibenjawong et al., 2011).

In the hospital environment, *S. aureus* has been recovered from bed linens (Boyce et al., 1997), patient gowns (Boyce et al., 1997), the floor (Boyce et al., 1997), overbed tables (Boyce et al., 1997), mattresses (Sexton et al., 2006), blood pressure cuffs (Boyce et al., 1997), and the ambient air (Sexton et al., 2006), among other surfaces. Studies have linked improved cleaning practices with reduced risk of acquiring antibiotic-resistant *S. aureus* infections in the hospital environment (Dancer, 2009; Rampling et al., 2001). Indeed, stringent infection control practices, including hand and hospital hygiene, are credited for the low rate of hospital-acquired MRSA infections in several Northern European countries (Borg et al., 2014).

The household environment may also play a role in recurrent *S. aureus* nasal colonization and skin and soft tissue infection (Davis, Iverson, et al., 2012; Fritz et al., 2014), although the directionality of the exchange of *S. aureus* between humans and their household environments is often unclear (Milstone, 2014). Some case reports of *S. aureus* infection indicate successful treatment only after decolonization of household surfaces and/or household pets (Ferreira et al., 2011; Kniehl et al., 2005; Masterton et al., 1995). In the household environment, *S. aureus* has been recovered from touch sites (e.g. TV remotes, game controllers, microwave handles), sites with heavy face and nose contact (e.g. pillows, sheets, towels), and air deposition sites (e.g. top of refrigerator, back of entertainment unit) (Davis, Iverson, et al., 2012). The household environment is thought to serve as reservoir for epidemic *S. aureus* strains, including USA300 (Uhlemann et al., 2011). There is some evidence that epidemic strains can survive longer in the environment than sporadic strains (Wagenvoort et al., 2000).

Detection and molecular typing

<u>Detection</u>

S. aureus is usually detected through a combination of identification via *S. aureus*-specific culture media, biochemical testing, and molecular detection of *S. aureus*-specific genes.

Commonly-used media that are selective and differential for *S. aureus* growth include: Baird Parker with Egg Yolk Tellurite Enrichment (multiple manufacturers), BBL™ CHROMagar™ Staph aureus (Beckton, Dickinson and Company, Franklin Lakes, NJ), Mannitol Salt Agar (multiple manufacturers), Vogel-Johnson agar (multiple manufacturers), and RAPID Staph (Bio-Rad Laboratories, Hercules, CA).

Biochemical identification of *S. aureus* usually involves a combination of gram-positive staining (all gram-positive bacteria produce a positive result), catalase testing (catalase catalyzes the decomposition of H_2O_2 to water and oxygen and is produced by all staphylococci), and coagulase testing (coagulase binds fibrinogen in the blood and converts it to insoluble fibrin; among staphylococci, only *S. aureus* produces coagulase).

Molecular detection of *S. aureus*-specific genes is considered the gold standard for *S. aureus* identification. Genes that are often targeted include: a factor essential for the expression of methicillin-resistance (*femA*), surface protein A (*spa;* encodes a cell-wall bound protein involved in increased pathogenicity), staphylococcal nuclease (*nuc*; encodes an enzyme involved in cleavage of the phosphodiester bonds between the nucleotide subunits of nucleic acids), and clumping factor A (*clfA*; encodes a surface protein that promotes binding to blood clots and traumatized tissue). Among confirmed *S. aureus*, molecular detection of the *mecA* gene is the gold standard for MRSA identification.

<u>Molecular Typing</u>

Molecular typing allows researchers to track the dissemination of *S. aureus* and MRSA strains into local, regional and global populations. The typing methods that will be discussed here are: multilocus sequence typing (MLST), multilocus variable number tandem repeat analysis (MLVA), and typing of the variable tandem repeat region of surface protein A (*spa* typing), although multiple other typing methods exist. These methods are highlighted because they tend to be highly discriminatory and are used globally.

Multilocus Sequence Typing. MLST is a relatively new typing method that is useful for examining the dissemination of strain types on a regional, national, and global scale (Maiden et al., 1998). MLST involves sequence analysis of 0.5-kb internal fragments of seven *S. aureus* housekeeping genes: *arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi*, and *yquiL*. Differences in these sequences correspond to distinct alleles for each housekeeping gene. Each allele is assigned a unique number; any *S. aureus* isolate can be identified by its allele profile for these seven genes, also known as its “sequence type.” Currently, there exists one global database containing all known sequence types (http://mlst.net), to which any researcher can upload new alleles. Global epidemiological investigations of MRSA and MSSA infection are thus possible using MLST. Unfortunately, MLST is time-consuming and expensive.

MLST’s greatest utility is its ability to provide insight into the clonal evolution of *S. aureus*. *S. aureus* strains are grouped into the same “clonal complex” (CC) when 5 out of their 7 housekeeping genes have identical sequences. The founder of a CC is calculated as the ST that differs from the largest number of other STs at only a single locus, rather than the ST that is detected most frequently (as this is subject to sampling bias). This methodology for determining a CC founder takes into account the way clones actually emerge and diversify. Subgroup founders are single-locus or double-locus variants of a CC founder. These variants may become prevalent in the population, and may diversify independently to produce their own set of single-locus and double-locus variants (Deurenberg et al., 2007). A constantly updated diagram depicting all known clonal complexes and their relationships to one another is available at http://eburst.mlst.net.

Multilocus variable number tandem repeat analysis. MLVA is a more discriminatory typing method than MLST and is also significantly cheaper, but suffers from a lack of interlaboratory reproducibility. MLVA involves the amplification of several conserved loci (usually 5+), each comprising tandem repeats. The number of base pairs in a single tandem repeat (known as the *length* of a tandem repeat) varies depending on which loci the repeat occurs in; the length of tandem repeats found in each locus to be amplified is determined ahead of time via sequence analysis. Following PCR amplification, the total length of each locus is assessed using gel or capillary electrophoresis. Because the length of repeats within each locus has previously been assessed, the number of repeats with an amplified locus can therefore be determined; this number corresponds to a unique allele number. A *S. aureus* isolate can be identified by its allelic profile for the 5+ loci that have been amplified. The more loci that are amplified to comprise an allelic profile, the more discriminatory the typing protocol. Despite its discriminatory power, the utility of MLVA for international surveillance is limited as MLVA protocols for typing of *S. aureus* are not harmonized or standardized.

There have recently been efforts to standardize protocols for MLVA typing of *S. aureus*. A recent paper by Schouls *et al*. (2009) describes a rapid MLVA protocol involving amplification of 8 loci via two multiplex PCRs, DNA sequencing, and allele assignment by software (Schouls et al., 2009). The method described proved equally as discriminatory as pulse-field gel electrophoresis (PFGE) and allowed for clustering of strains into clonal complexes that compared well with MLST clonal complexes. However, widespread acceptance of this protocol may be hampered as other MLVA protocols for *S. aureus* typing continue to be published in the literature (Pourcel et al., 2009; Sobral et al., 2012).

***Spa* typing.** *Spa* typing is more discriminatory than MLST and similar in discriminatory power to MLVA. It may be much less costly than both methods since it involves sequencing of only a single locus. The gene encoding surface protein A is unique to *S. aureus* and contains a polymorphic region X, which is characterized by one to 25 24-bp tandem repeats. Diversity in repeats is attributed to duplications of repeats, deletions, and less commonly, mutations (Deurenberg et al., 2007). However, the first repeat in region X always begins with the sequence GAG, while subsequent repeats always begin with AAA (M. Sørum, personal communication, November 26, 2011). Sequencing the *spa* gene reveals how many tandem repeats are present in a strain, which repeats they are, and in what order they occur; this corresponds to a certain *spa* type. Researchers can identify *spa* types as well as upload novel *spa* types using a central *spa* server, available at http://www.spaserver.ridom.de. Because of its simplicity, low cost, and the fact that it can be performed quickly using “in-house” technologies, this typing method is used widely in hospitals and research labs (Deurenberg et al., 2007; Sørum, 2011).

Proposed markers of livestock association

There are currently no established markers for livestock-associated *S. aureus*. Several have been proposed, including clonal complex, absence of the bacteriophage-encoded staphylococcal complement inhibitor (*scn*) gene, and tetracycline resistance.

Since its initial detection among humans in contact with livestock, CC398 has commonly been referred to as "livestock-associated." However, phylogentic evidence indicates two distinct reservoirs of CC398: livestock-adapted CC398, which primarily circulates among pigs, and livestock-independent CC398, which is primarily transmitted among humans (Price et al., 2012). Phylogentically distinct human and livestock clades have been detected among other CCs as well, including CC5, CC97, and CC8 (Lowder et al., 2009; Resch et al., 2013; Spoor et al., 2013). These findings indicate that while clonal complex may be a useful indicator of livestock-association, it cannot be used as a sole marker; other indicators are needed in combination with CC to distinguish livestock-adapted from human-adapted isolates.

CC9 is the most commonly recovered strain type from pigs and pig workers in multiple Asian countries (Chuang & Huang, 2015; Larsen et al., 2012; Patchanee et al., 2014), and is frequently detected among hog CAFO workers in eastern North Carolina (Nadimpalli et al., 2014b; Rinsky et al., 2013). Phylogenetic analysis of a global collection of human and animal CC9 isolates has not been conducted; thus, it is unclear whether *S. aureus* CC9 recovered from the livestock environment is truly livestock-adapted, or whether these bacteria reflect widespread circulation of a human-adapted clone among animals. Still, the unusual dominance of this strain type among livestock and individuals in contact with livestock in multiple countries merits consideration of CC9 as a presumptive indicator of livestock association.

Among studies that have examined *S. aureus* CCs with phylogentically distinct human and livestock clades, absence of the bacteriophage-encoded *scn* gene has been detected almost universally (99-100%) among livestock-associated isolates (Lowder et al., 2009; Price et al., 2012; Resch et al., 2013; Spoor et al., 2013). *scn* and other phage-encoded genes *sak* and *chp* comprise the human immune evasion cluster (IEC), which can make *S. aureus* strains highly pathogenic among humans. The IEC provides no clear advantage among *S. aureus* circulating among livestock, which may explain its loss among livestock-adapted isolates. The speed with which loss occurs is unknown, as are the factors that might induce this loss, besides antibiotic use (Allen et al., 2011). Thus, absence of *scn* may be a useful indicator of livestock-association in combination with CC and/or other indicators (Rinsky et al., 2013).

Tetracycline is heavily used in food animal production in the United States (Love et al., 2011). Tetracycline resistance has been identified in the literature as a marker of livestock-association among isolates belonging to CC398 (Price et al., 2012), but not among other CCs. However, recent evidence suggests that phenotypic tetracycline resistance co-occurs frequently with absence of the *scn* gene, even beyond the scope of CC398 . Although further research is needed, this finding suggests that tetracycline resistance may be a more broadly-applicable indictor of livestock-associated *S. aureus*.

Routes for exposure to *S. aureus* associated with food animal production

Occupational exposure

Industrial food animal production workers may be exposed to a variety of physical, biological, and chemical occupational hazards (Cole et al., 2000). These include (but are not limited to): high temperatures; loud noises; physical injuries resulting from animal interactions or repetitive tasks; bioaerosols and dust resulting from animals, feces, and feed; and gases, vapors, and chemicals from pesticides and decomposing waste. Some occupational hazards, including inhalation or ingestion of bioaerosols and injuries through animal interaction, may result in exposure to zoonotic pathogens. Although personal protective equipment (PPE) such as gloves, boots, and face masks is recommended to minimize inhalation, ingestion, and dermal exposures, worker use is not consistent and may not be remedial once symptoms have already begun (Cole et al., 2000).

Bioaerosols

Within confinement buildings, workers can be exposed to organic dust generated from animal dander, feces, and feed; airborne microbial pathogens; and microbe-bearing particulates, known collectively as “bioaerosols.” Airborne microbial pathogens and microbe-bearing particulates originate from fecal shedding, decomposing waste (Nehme et al., 2008) and animal activity (e.g., coughing and sneezing) (Cole et al., 2000). Bioaerosols ranging from 1-5 μm in size can be inhaled or swallowed and 1-2 μm particles can be retained in the lung alveoli; thus, bioaerosols of these diameters pose the greatest health risk to livestock operation workers (Salem & Gardner, 1994). Numerous microbial pathogens have been identified in the ambient air of confinement buildings, including bacteria belonging to the species *Enterococcus*, *Streptococcus*, *Bacillus*, *Listeria*, *Moraxella*, *Acinetobacter*, *Pseudomonas*, *Nocardia*, *Lactobacillus*, *Clostridia,* and *Staphylococcus* (Chapin et al., 2005; Cormier et al., 1990; Gibbs et al., 2006; Nehme et al., 2008; Predicala et al., 2002; Sapkota et al., 2006; Scarpino & Quinn, 1998). Gram-positive bacteria such as *Enterococcus* and *Streptococcus* are often dominant in this environment (Cole et al., 2000).

Direct animal-to-human transmission

Food animal production operation workers may also be exposed to microbial zoonotic pathogens as a result of direct contact with animals. Workers may come into direct contact with animal fluids as a result of administering injections or medications, tail docking, performing castrations, or inseminating sows. These tasks along with others, such as cutting teeth or moving animals, may result in dermal abrasions that could provide entry for microbial pathogens. Zoonoses related to industrial livestock production that may be transmitted to workers via this exposure pathway (although not exclusively via this pathway), include *Yersinia enterocolitica*, *Toxoplasma gondii, Salmonella* spp., *Leptospira* spp., *Erysipelothrix* *rhusiopathiae*, *Brucella suis*, *Campylobacter, Streptococcus suis*, Hepatitis E virus, and influenza virus (Cole et al., 2000; Gilchrist et al., 2007; Myers et al., 2006).

Environmental exposure

Individuals living in regions with a high density of CAFOs may be environmentally exposed to zoonotic pathogens through bioaerosol emissions (Ferguson, 2012; Gibbs et al., 2006; Green et al., 2006), direct spraying of liquid manure (Casey, Curriero, et al., 2013), contaminated soil (Schulz et al., 2012), and contaminated insects or other pests (Ahmad et al., 2011; Van De Giessen et al., 2009). Extreme weather events (e.g. flooding) may exacerbate some of these exposures (Wing et al., 2002).

Several studies have observed higher rates of infection with antibiotic-resistant *S. aureus* among individuals living in regions with a high density of hog operations, compared to the general population (Feingold et al., 2012; Lekkerkerk et al., 2015; Van Rijen et al., 2014). Some have speculated that an environmental route of exposure may be responsible for increased infection rates (Casey, Curriero, et al., 2013; Feingold et al., 2012), although no study published thus far has examined this hypothesis directly. Children (Febriani et al., 2009), pregnant women, and elderly persons living proximal to CAFOs may be at particular risk for infection with zoonotic pathogens transmitted via environmental pathways.

<u>Bioaerosols</u>

Bioaerosols produced inside confinement buildings are exhausted into the surrounding environment through ventilation fans in order to maintain ambient dust levels and a suitable temperature for the animals. The distance and concentration of bioaerosols downwind of a CAFO may depend on: a) wind direction and velocity, b) ambient temperature and humidity, c) the amount of UV light, and d) the environmental hardiness of microorganisms comprising the bioaerosols (National Risk Management Laboratory, 2004). Studies in the United States have observed airborne *S. aureus* plumes downwind of industrial hog operations up to 215 meters (Ferguson, 2012; Gibbs et al., 2006; Green et al., 2006), although no study has tracked airborne

S. aureus emissions across increasing distances to the point of non-detection. Other airborne microorganisms observed downwind of confinement buildings include *Salmonella* spp. and fecal coliforms (Gibbs et al., 2004; Green et al., 2006).

Direct spraying of liquid manure

In North Carolina, liquid manure produced on CAFOs is flushed into storage lagoons, then periodically sprayed onto nearby fields for disposal. Zoonotic pathogens present in liquid manure can enter surrounding streams and water bodies through runoff or could be aerosolized and dispersed via the spraying process. In North Carolina, hog CAFOs and lagoons built after 1995 are required to be at least 1,500 feet from occupied homes, 2,500 feet from schools, hospitals, or churches, and at least 500 feet from any property boundary (North Carolina General Assembly, 1995); however, most hog CAFOs operating in North Carolina were built before 1995 (due to a 1997 moratorium on permitting new hog CAFO liquid waste storage lagoons). Community residents living near hog CAFOs in eastern North Carolina have reported liquid manure sprayed directly onto their residential property, including on homes, cars, and laundry hanging to dry (Wing, 2002). Living proximal to hog manure spray fields has been linked to an increased risk for *S. aureus*, MRSA, and skin and soft tissue infection (Casey, Curriero, et al., 2013).

Contamination by soil, insects, or other pests

Soil near and downwind of CAFOs may be contaminated with zoonotic bacteria that can be tracked into the household environment by humans, pets, or pests. Soils on which liquid manure is disposed can be contaminated with bacteria and antibiotic-resistance genes (Chee-Sanford et al., 2009). *Staphylococcus* spp., *Clostridium perfringens*, and fecal coliforms have been observed at higher levels in soils frequently irrigated with liquid hog manure compared to nearby soils that have not been sprayed (Mclaughlin et al., 2010). A recent study suggests that fields that have not been irrigated with manure may also become contaminated through deposition of airborne bacteria emitted from confinement barns' ventilation systems (Schulz et al., 2012).

Flies (Ahmad et al., 2011; Graham et al., 2009), cockroaches (Ahmad et al., 2011), rats (Van De Giessen et al., 2009), and other pests detected on or near CAFOs can carry antibiotic-resistant *S. aureus* and other bacteria in their digestive tracts or on their exterior surfaces. Insects and pests may transfer antibiotic-resistant bacteria from CAFO barns and lagoons to proximal households through physical contact or fecal shedding. Previous work has established a link between density of house fly and cockroach populations and household-level incidence of foodborne illness (Ahmad et al., 2011). Community residents living near hog CAFOs in eastern North Carolina have described high concentrations of flies in and around their homes (Tajik et al., 2008).

Surveillance studies among food animal production workers

Surveillance of pathogen exposure among food animal production workers allows for early detection of potentially novel zoonoses and the implementation of measures to prevent transmission. Several European countries survey *S. aureus* nasal carriage and/or infection among those accessing health care; surveillance for neither *S. aureus* nasal carriage nor *S. aureus* infection occurs on a national scale in the United States.

Surveillance methods

There are two main methods for pathogen surveillance: (a) passive, healthcare provider-based surveillance, also known as "sentinel surveillance," and (b) active, population-based surveillance. Both methods of surveillance are critical to guiding the public health response to emergent zoonotic pathogens.

Passive surveillance requires health care providers to report cases of specific illnesses to a centralized database or public health organization. Samples may also be submitted to a centralized organization as part of passive surveillance. Passive surveillance is advantageous as it can often easily be integrated into routine activities and requires minimal resources compared to other surveillance methods. However, there are several disadvantages. First, passive surveillance only detects symptomatic cases that are severe enough to prompt an individual to seek health care. Therefore, mild or non-symptomatic exposures to zoonotic pathogens could easily be missed. Second, the effectiveness of passive surveillance is incumbent on the assumption that persons with illness will seek health care. However, access to health care is limited for many populations in the US (particularly in rural areas), meaning many symptomatic cases may go unreported. Third, because cases are not reported from a clearly defined population, it is difficult to calculate population prevalence and disease burden estimates.

Population-based surveillance involves active surveillance of a cohort for exposure to a specific pathogen(s). While more costly and resource-intensive, there are numerous advantages to population-based surveillance compared to passive surveillance. First, exposures to zoonotic pathogens can be detected among persons who may not normally seek health care (whether due to time constraints, financial constraints, or cultural preferences). Second, active surveillance can identify mild or non-symptomatic exposures to zoonotic pathogens. Third, because the population from which cases are identified is well defined, it is possible to calculate pathogen burden, prevalence of infection, and to assess household transmission dynamics. Often, some or all of these parameters must be evaluated in order to determine whether measures should be implemented to prevent transmission. Despite these advantages, population-based surveillance may not be sustainable in the long-term without stable financial and political support.

Surveillance for *S. aureus* in the United States and globally

Passive surveillance of *S. aureus* nasal carriage and infection is conducted among those accessing health care in several European countries, including Denmark (by the Statens Serum Institut), the Netherlands (by the National Institute for Public Health and Environment), Finland (by the National Institute for Health and Welfare), and Sweden (by the Swedish Institute for

Infectious Disease Control). Data on employment in livestock production operations is often collected as part of passive surveillance protocols. Numerous active surveillance studies of *S. aureus* exposure among clearly defined cohorts of livestock workers have also been conducted in several European countries (Denis et al., 2009; Geenen et al., 2013; Huber et al., 2010; Morcillo et al., 2012).

To the best of our knowledge, passive surveillance of *S. aureus* nasal carriage and infection does not occur on a national scale in the United States. Passive surveillance of *S. aureus* nasal carriage and infection is conducted by some individual health care providers, including providers in North Carolina, but data collection is largely for on-site infection control and is not reported to a centralized database or public health organization. Only one active surveillance study of *S. aureus* exposure has been conducted in the United States among a clearly defined cohort of livestock workers (Smith et al., 2009) .

Public health risks associated with livestock-associated *S. aureus*

Livestock-associated *S. aureus* and MRSA are responsible for a growing number of infections in several European countries, including the Netherlands, Germany, and Denmark (Smith et al., 2011; Statens Serum Institut et al., 2010). However, because livestock-associated *S. aureus* tend to lack human-specific genes and MGEs that are involved in invasive human infection (Price et al., 2012), the percent of extremely serious infections (such as bacteremia) caused by livestock-associated *S. aureus* and MRSA is low (Köck, Brandt, et al., 2012). Transmission of livestock-associated *S. aureus* between humans is also relatively rare, even within the hospital setting (Wassenberg et al., 2011).

The magnitude of the public health risk posed by livestock-associated *S. aureus* may depend on the ability of these bacteria to persistently colonize the human nose. Previous work suggests that any *S. aureus* strain capable of colonizing the human nose can cause invasive infections given the proper circumstances (e.g. acquisition of virulence factors) (Neela et al., 2009), and persistent nasal carriage of MRSA has been linked to an increased risk of infection in the clinical setting (Wertheim et al., 2005). However, few studies have examined persistence of carriage of livestock-associated *S. aureus*, and those conducted have yielded differing results (Frana et al., 2013; Graveland et al., 2011; Köck, Loth, et al., 2012; Van Cleef et al., 2011; Verkade et al., 2013). An experimental study has shown that livestock-associated *S. aureus* can persist in the human nose for at least as long as a human-adapted strain (7 to 21 days for the human strain used), and that livestock-associated *S. aureus* can successfully compete with human-adapted *S. aureus* as a nasal colonizer (Slingerland et al., 2012). However, observational studies assessing individuals with short-term exposure to livestock (e.g. visiting students, researchers) report that livestock-associated MRSA do not persist in the human nose after 24 hours of non-exposure to colonized animals (Frana et al., 2013; Van Cleef et al., 2011). Conversely, individuals with more frequent exposure to livestock (e.g. farmers, veterinarians) demonstrate inconsistent results including persistent carriage for up to two years (Köck, Loth, et al., 2012; Verkade et al., 2013), and some loss of carriage following periods of low or no exposure to animals (13 days on average) (Graveland et al., 2011). No studies have examined persistence of livestock-associated *S. aureus* among workers at CAFOs in North Carolina, where most workers are exposed to intensively-raised livestock >50 hours/week, with no more than one to two days off per month (see Chapter 2). Further studies evaluating the potential for livestock-associated *S. aureus* to persistently colonize the human nose must be conducted in order for the public health risk of exposure to these bacteria to be evaluated.

Public health significance

Antibiotic-resistant *Staphylococcus aureus* is a pathogen of clinical and public health concern to which industrial food animal production workers may be occupationally exposed. Little is known about occurrence or persistence of exposure to *S. aureus* among industrial livestock workers in North Carolina, a region with intensive hog growing operations, or about dissemination of *S. aureus* into workers' households. In the United States and globally, further epidemiological investigation is needed to identify targeted intervention measures that could reduce or eliminate exposure among livestock workers, who can act as a bridging population for cross-species exposure, adaptation, and transmission of zoonotic pathogens to the community. The proposed research will fill these knowledge gaps by using a repeated-measures study design to elucidate persistence of exposure to zoonotic *S. aureus* among industrial livestock workers and their household members. Livestock workers in North Carolina are primarily poor, nonwhite, non-citizens with little access to health care, precluding passive surveillance of *S. aureus* and among this population. Thus, the proposed work will also provide a first insight into the prevalence of infections with *S. aureus* among workers and their household members in North Carolina. More broadly, this work will add to the growing body of literature examining the public health implications of large-scale production of hogs on confined industrial operations.

CHAPTER TWO: PERSISTENCE OF LIVESTOCK-ASSOCIATED ANTIBIOTIC-RESISTANT *STAPHYLOCOCCUS AUREUS* AMONG INDUSTRIAL HOG OPERATION WORKERS IN NORTH CAROLINA OVER 14 DAYS[1]

SUMMARY

Objective: This study aimed to evaluate the persistence of nasal carriage of *Staphylococcus aureus*, methicillin-resistant *S. aureus* and multidrug-resistant *S. aureus* over 14 days of follow-up among industrial hog operation workers in North Carolina. Methods: Workers anticipating at least 24 hours away from work were enrolled June–August 2012. Participants self-collected a nasal swab and completed a study journal on the evening of day 1, and each morning and evening on days 2–7 and 14 of the study. *S. aureus* isolated from nasal swabs were assessed for antibiotic susceptibility, *spa* type and absence of the *scn* gene. Livestock association was defined by absence of *scn*. Results: Twenty-two workers provided 327 samples. *S. aureus* carriage end points did not change with time away from work (mean 49 h; range >0–96 h). Ten workers were persistent and six were intermittent carriers of livestock-associated S. aureus. Six workers were persistent and three intermittent carriers of livestock-associated multidrug-resistant *S. aureus*. One worker persistently carried livestock-associated methicillin-resistant *S. aureus*. Six workers were non-carriers of livestock-associated *S. aureus*. Eighty-two per cent of livestock-associated *S. aureus* demonstrated resistance to tetracycline. A majority of livestock-associated *S. aureus* isolates (n=169) were CC398 (68%) while 31% were CC9. No CC398 and one CC9 isolate was detected among scn-positive isolates. Conclusions: Nasal carriage of livestock-associated *S. aureus*, multidrug-resistant *S. aureus* and methicillin-resistant *S. aureus*

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can persist among industrial hog operation workers over a 14-day period, which included up to 96 hours away from work.

INTRODUCTION

Staphylococcus aureus is an opportunistic pathogen and an important cause of global morbidity and mortality (Lowy, 1998). Most infections caused by *S. aureus* manifest as skin and soft tissue infections, but invasive and sometimes fatal disease is also possible.

Some strains of *S. aureus* are adapted to colonize and infect livestock and poultry. Livestock-associated strains of *S. aureus*, including methicillin-resistant (MRSA) and multidrug-resistant (MDRSA) strains, can be transmitted to humans who are in close contact with livestock (Harrison et al., 2013; Price et al., 2012). In 2005, a novel strain of livestock-associated MRSA belonging to multi-locus sequence typing clonal complex (CC) 398 was detected for the first time among hogs and hog farmers in France and the Netherlands (Armand-Lefevre et al., 2005; Voss et al., 2005). Nasal carriage of MRSA CC398 is now prevalent among individuals in contact with hogs and/or other livestock in many European countries (Smith et al., 2011), and has been detected among livestock workers in the United States (Rinsky et al., 2013; Smith et al., 2009) and Canada (Khanna et al., 2008) as well.

Understanding the persistence of nasal carriage of livestock-associated *S. aureus* among individuals regularly exposed to livestock is essential in determining the magnitude and severity of the public health risk posed by the dissemination of these bacteria. However, studies examining this outcome have yielded conflicting results. Some epidemiological studies have found that nasal carriage of livestock-associated *S. aureus* is transient among those with sporadic, short-term exposure to livestock (e.g. visiting students, field workers), and that these bacteria are unable to persist in the human nose after periods of low or no exposure to colonized or infected animals (Frana et al., 2013; Van Cleef et al., 2011). However, other studies assessing individuals with more frequent exposure to livestock (e.g. farmers, livestock workers, veterinarians) have found that many individuals colonized with livestock-associated *S.*

aureus remain positive after periods of low to no exposure (Graveland et al., 2011; Köck, Loth, et al., 2012; Verkade et al., 2013). A laboratory-based study has also determined that livestock-associated *S. aureus* can persist in the human nose for at least as long as a human-adapted, community-associated strain of *S. aureus*, and that these strains can successfully compete with human-associated *S. aureus* as a nasal colonizer (Slingerland et al., 2012).

There are several additional gaps in our present understanding of the persistence of livestock-associated *S. aureus* among individuals exposed to livestock. First, the majority of published studies examining persistence of livestock-associated *S. aureus* have focused exclusively on the presence and absence of CC398, although other markers of livestock-association (including absence of the phage-encoded *scn* gene (Sung et al., 2008; Verkaik et al., 2011) and specific resistance patterns (Price et al., 2012)) may be present among bacteria transmitted from livestock to workers. Additionally, most studies have only examined persistence of nasal carriage of MRSA (Frana et al., 2013; Graveland et al., 2011; Köck, Loth, et al., 2012; Van Cleef et al., 2011), although livestock-associated MDRSA is known to be present among livestock workers and also has important implications for clinical and public health (Rinsky et al., 2013). Furthermore, in the United States specifically, the question of persistence of carriage of livestock-associated *S. aureus* has not been well-examined. One published study has examined persistence of nasal carriage of MRSA CC398 among individuals sporadically exposed to livestock (Frana et al., 2013), but no study has examined persistence of carriage among those with more frequent and prolonged exposure, such as industrial livestock workers.

To begin to address these research gaps, we conducted a 14-day study examining the temporal dynamics of nasal carriage of livestock-associated *S. aureus*, MRSA, and MDRSA among 22 workers employed at industrial hog operations in North Carolina. Here, we present information regarding 1) genetic and phenotypic characteristics of *S. aureus* detected; 2) persistence of *S. aureus* nasal carriage before, during, and after time away from work at an industrial hog operation; and, 3) whether carriage states (persistent, intermittent, or non-carriage) were associated with personal or occupational exposures.

METHODS

Data were collected between June and August 2012 in North Carolina by researchers from the University of North Carolina at Chapel Hill (UNC) with community organizers from the Rural Empowerment Association for Community Help (REACH). The UNC Public Health-Nursing institutional review board approved this study (IRB: 12-0712). Before participating, workers provided written informed consent.

Data Collection

Community organizers from REACH recruited volunteers who fit the following inclusion criteria: worked at an industrial hog operation; resided in North Carolina; were at least 18 years old; could speak and read English or Spanish; had access to a refrigerator; and anticipated at least 24 hours away from work during the first seven days of the study. Participants were enrolled in four cycles. Upon enrollment, participants responded to a baseline questionnaire administered by the community organizer. The baseline questionnaire assessed demographic information, household member characteristics, pet ownership, work activities, medical history, and risk factors for exposure to *S. aureus*, including MRSA. Because of concerns about privacy and confidentiality with respect to employment, no identifying information about livestock operations was collected.

On day one of the 14-day study, each cycle of participants attended a training session where study protocols were reviewed and participants received instruction about how to complete study activities. After receiving instruction from researchers, participants self-collected a baseline nasal swab during the training session. For the next six days of the study, participants self-collected a nasal swab in the morning and in the evening, regardless of whether or not they worked at their job at an industrial hog operation that day (**Appendix A, Figure A1**). Participants also self-collected a nasal swab in the morning and in the evening on the 14th day of the study. In total, up to 15 swabs were collected per participant. At the same time each swab was collected, participants recorded information about exposures, symptoms, and work activities, including time away from work, in a study journal. Community organizers regularly checked in with participants during the 14-day period to answer questions and assist with data collection (**Appendix A, Figure A1**).

Detection of *S. aureus* and MRSA

Baseline nasal swabs were transported to UNC at 4°C within 24 hours of collection. Swabs collected between the second and 14th day of the study were stored in participants' refrigerators and picked up on the 8th and 14th day of study by a REACH community organizer. These swabs were transported to UNC at 4°C within eight days of participant self-collection. An experiment was conducted prior to beginning this study to confirm survival of *S. aureus* on the swabs during an eight-day holding period (**Appendix A**).

Upon arrival in the laboratory, swabs were inoculated into 10 ml of Mueller-Hinton broth containing 6.5% NaCl, then incubated overnight at 37°C. To isolate presumptive *S. aureus*, a loopful of Mueller-Hinton broth was streaked onto Baird Parker and CHROMagar™ Staph aureus media (BD, Franklin Lakes, NJ) and incubated at 37°C for 24 hours. Both media were used in parallel to increase detection of *S. aureus* from our study population (Nadimpalli et al., 2013). Colonies with morphological characteristics of *S. aureus* were confirmed through catalase testing, tube coagulase testing with rabbit plasma (BD BBL™, Franklin Lakes, NJ), and multiplex PCR detection of a *Staphylococcus*-specific region of the 16S rRNA gene and the *S. aureus*-specific *nuc* gene (Poulsen et al., 2003). *S. aureus* isolates that were positive for *mec*A were classified as MRSA.

Approximately one-third of swabs collected were not immediately processed upon arrival at UNC due to time and labor constraints. Immediately upon receipt, these swabs were shipped overnight at 4°C to Johns Hopkins University (JHU) and archived in tryptic soy broth with 20% glycerol (w/v) at -80°C. After approximately four months of storage at -80°C, swabs were thawed and assessed for *S. aureus* and MRSA using the same procedures as above. Laboratory protocols and training of personnel were identical in both labs. We observed no systematic differences in the data produced.

Antibiotic susceptibility testing

One isolate from each *S. aureus*-positive nasal swab was assessed for susceptibility to 12 classes of antibiotics: aminoglycosides, β-lactams, cephalosporins, floroquinolones, glycopeptides, lincosamides, macrolides, oxazolidones, rifamycin, streptogramins, sulfonamide/methoprim, and tetracyclines (see **Appendix A: Table A2** for a listing of antibiotics used). Among *S. aureus* isolates identified at UNC-Chapel Hill (n=151), the Kirby-Bauer disk diffusion method was used to assess susceptibility to all given antibiotic classes except glycopeptides (assessed using brain heart infusion agar supplemented with 5 mg/L teicoplanin (Fitzgibbon et al., 2007)). Diameter interpretations were based on Clinical and Laboratory Standards Institute (CLSI) guidelines (Clinical and Laboratory Standards Institute, 2010). Inducible clindamycin resistance was evaluated in erythromycin-resistant isolates using the D-zone test (Steward et al., 2005). Among *S. aureus* isolates identified at Johns Hopkins University (n=67), the Phoenix Automated Microbiology System (BD Diagnostic Systems, Sparks, MD) was used to assess susceptibility to all 12 antibiotic classes (Carroll et al., 2006). Testing was completed by the Clinical Microbiology Laboratory at the Johns Hopkins Hospital.

S. aureus that demonstrated complete resistance to three or more classes of antibiotics were classified as MDRSA (Magiorakos et al., 2012). MRSA isolates meeting the definition of MDRSA were classified as multidrug-resistant MRSA.

Molecular analyses

The staphylococcal protein A (*spa*) gene was amplified and sequenced for one isolate from each *S. aureus*-positive nasal swab using methods described previously.(European Union Reference Laboratory for Antimicrobial Resistance, 2009). All isolates were characterized by *spa* typing using the Ridom Staph Type standard protocol (http://www.ridom.com) and the

Ridom SpaServer (http://spa.ridom.de/index.shtml). The putative clonal complex (CC) to which each *spa* type belonged was inferred based on the existing literature. Clonal complexes are groups of closely-related strains that likely evolved from a single founder; CC can provide insight into the epidemiology and geographic origin of a specific strain. Isolates that could not be assigned to a putative CC with a high degree of certainty based on *spa* type and the existing literature alone were additionally analyzed by multilocus-sequence typing (MLST) (Enright et al., 2000). For these isolates, CCs were determined using eBURST (version 3; http://eburst.mlst.net) and the stringent group definition (6/7 shared alleles) (Feil et al., 2004).

We used PCR to determine whether the *scn* gene was absent from *S. aureus* isolates (Van Wamel et al., 2006). We also used PCR to determine whether the *tet*(M) gene was present among *S. aureus* isolates, as *tet*(M) is a proposed marker of livestock association among CC398 isolates, specifically (Warsa et al., 1996). Both targets were assessed simultaneously in one isolate from each *S. aureus*-positive nasal swab using a novel duplex PCR assay (the same isolate for which we tested antibiotic susceptibility) (Stegger et al., 2013).

Assessment of livestock-associated *S. aureus*

There are currently no established markers for livestock-associated *S. aureus*. Although CC398 is commonly used, this marker may not be specific or sensitive in identifying livestock-associated isolates (Price et al., 2012). However, studies that have examined *S. aureus* CCs with phylogentically distinct human and livestock clades (e.g. CC398, CC5, CC97, or CC8) have detected near universal loss of *scn* (99-100%) among livestock-associated isolates (Lowder et al., 2009; Price et al., 2012; Resch et al., 2013; Spoor et al., 2013). Thus, we considered absence of *scn* to be a proxy for livestock association among all *S. aureus* isolates.

Once stratified by livestock association (absence of *scn*), we examined the distribution of tetracycline resistance and CC among all *S. aureus* isolates. Tetracycline is used heavily in food animal production in the United States (Love et al., 2011). Tetracycline resistance (typically mediated by the *tet*(M) gene) has been identified by others as a marker of livestock association among isolates belonging to CC398 (Price et al., 2012) but not among other CCs (Love et al., 2011).

Statistical analysis

Based on nasal carriage definitions used by van Belkum *et al*. (Van Belkum et al., 2009), we defined persistent carriage with *S. aureus*, MRSA, MDRSA, and *scn*-negative *S. aureus* as positivity for the outcome of interest for all, or all but one, collected nasal swab (i.e., 15/15 or 14/15); intermittent carriage as positivity for 1/15 to 13/15 collected nasal swabs; and non-carriage as negativity for the outcome for all collected nasal swabs. Our definition of persistent carriage allowed for one negative swab in order to minimize misclassifications resulting from laboratory or sampling error (Van Belkum et al., 2009). Baseline swabs for three participants could not be analyzed due to contamination during collection, resulting in 14 collected swabs (instead of 15). The aforementioned definitions were adjusted accordingly for these individuals.

We described the distribution of personal characteristics (age, gender, education, use of antibiotics, recent hospitalization, participation in contact sports) (Cohen, 2008; Gorwitz et al., 2008; Mainous et al., 2006), household characteristics (number and age of household members, pets, location of home on a hog operation) (Davis, Iverson, et al., 2012; Uhlemann et al., 2011), and work exposures (time at job, average hours worked/week, number and age of hogs in contact with at work, and time since last work shift) within the study population (Smith et al., 2011), focusing on potential risk-factors related to carriage of antibiotic-resistant *S. aureus*. Ever carriage of each type of *S. aureus* was calculated as the number of workers who carried the type at any time during the study period. We also calculated the mean daily prevalence of carriage of each *S. aureus* outcome by averaging the number of carriers each day. Using the aforementioned definitions we calculated the proportions of persistent, intermittent, and non-carriers of each outcome over the study period. We also examined the distribution of antibiotic resistance patterns and genetic markers of *S. aureus* observed among participants.

To examine associations between demographic, behavioral, and work-related risk factors and carriage states (persistent, intermittent, and non-carriers) we generated crude odds ratios using tabular methods. Crude odds ratios were also examined using polytomous logistic regression.

RESULTS

Participant characteristics

Twenty-two industrial hog operation workers participated in the study, all of whom identified as Hispanic. Most participants were between 25-44 years of age (73%), had received at least a high school education (68%), and lived in households with three or more other people (86%) (**Table 2.1**). Twenty-three percent (5/22) reported living on the same property as the industrial hog operation for which they worked. One worker reported using antibiotics and another reported visiting a hospital within a month of enrollment (results not shown). Most participants reported working more than five days a week and more than eight hours a day, resulting in a majority (59%) of participants reporting working >50 hours during an average week. A majority of participants reported contact with sows, nursing, or weaned pigs at work (91%), although exposure to older pigs was reported by 7/22 workers (32%).

Occurrence of *S. aureus*, MRSA, and MDRSA

Eighty-six percent (19/22) of workers carried *S. aureus*, 5% (1/22) carried MRSA, and 46% (10/22) carried MDRSA during at least one sampling point of the 14-day follow-up (**Table 2.2)**. Mean daily prevalence of *S. aureus*, MRSA, and MDRSA was 65%, 4%, and 33%, respectively. Fifty-five percent (12/22), 5% (1/22), and 27% (6/22) were persistent carriers of *S. aureus*, MRSA, and MDRSA, respectively. The participant observed to persistently carry MRSA carried multidrug-resistant MRSA for 36% (5/14) of the sampling points. Occurrence of nasal carriage of *S. aureus,* MRSA, and MDRSA overlaid with time away from work is presented in **Figure 2.1**. Loss of carriage of *S. aureus,* MRSA, or MDRSA did not occur with time away from work (Mean: 49 hr; Range: 0-96 hr).

Antibiotic resistance phenotypes

Among the *S. aureus* isolates analyzed for this study, complete or intermediate resistance was observed to all of the 12 antibiotic classes tested except glycopeptides, oxazolidones, rifamycin, streptogramins, and sulfonamide/methoprim. We observed 12 distinct antibiotic susceptibility patterns among *S. aureus*-positive participants. The distribution of these patterns is depicted in **Appendix A: Table A4**. We observed few changes in within-participant patterns of resistance during the 14-day study period; changes in resistance patterns were typically concordant with changes in *S. aureus* genotype.

Distribution of *S. aureus* clonal complexes

We observed a diversity of clonal complexes among the 19 industrial hog workers who carried *S. aureus* at least once during the 14-day period (**Figure 2.2**). CC398 was most frequently detected - carried by seven workers persistently and one intermittently, followed by CC9 - carried by two workers persistently and seven intermittently. CC30, CC8, CC15, CC20, and CC779 were also observed, though less frequently. We observed a change in CC for 18% (4/22) of workers over the study period, and no change for the remaining participants (15/22). See **Appendix A: Table 3** for a listing of *spa* types, MLST results, and inferred CCs for all *S. aureus* isolates detected in this study.

Livestock-associated *S. aureus*, MRSA, MDRSA

We detected livestock-associated-*S. aureus* at least transiently among 73% (16/22) of participants (**Table 2.2**). Mean daily prevalence of livestock-associated *S. aureus*, livestock-associated MRSA, and livestock-associated-MDRSA was 51%, 4%, and 33%, respectively. During the study period, 10/22 (46%) workers were persistent and 6/22 (27%) were intermittent carriers of livestock-associated *S. aureus*. Of the 16 workers carrying livestock-associated *S. aureus* at least once, six workers were persistent and three were intermittent carriers of livestock-associated MDRSA. One persistent carrier and no intermittent carriers of livestock-associated MRSA were observed. Six of 22 (27%) workers were non-carriers of livestock-associated *S. aureus* over the study period. Neither time away from work nor time of sampling appeared to be associated with changes in carriage of livestock-associated *S. aureus*, livestock-associated MRSA, or livestock-associated MDRSA.

Detailed depictions of the distribution of observed CCs by absence of *scn* are provided in **Figures 2.2 and 2.3.** Only *scn*-negative *S. aureus* belonging to CC398, CC9, and CC20 were observed. All CC398 isolates (115/115), 98% of CC9 isolates (53/54), and 14% of CC20 isolates (1/7) were *scn*-negative. All MRSA (15/15) and most MDRSA isolates (106/110) were *scn*-negative. Additionally, 82% of *scn*-negative *S. aureus* isolates (138/169) were phenotypically tetracycline-resistant, while no *scn*-positive *S. aureus* isolates demonstrated tetracycline resistance (**Figures 2.2 and 2.3)**. Tetracycline resistance was associated with presence of *tet*(M) for 100% of *scn*-negative CC398 isolates (111/111) and 22% of *scn*-negative CC9 isolates (6/27) demonstrating phenotypic resistance to tetracycline (data not shown).

DISCUSSION

In this 14-day repeated measures study of industrial hog operation workers in North Carolina, we observed that 45.5% of participants were persistent carriers of livestock-associated *S. aureus*. We did not observe within-person associations between time away from work and the various *S. aureus* endpoints examined (including livestock-associated strains). To our knowledge, this is the first study in the United States to examine persistence of nasal carriage with livestock-associated *S. aureus* among workers employed at industrial livestock operations. The distribution of CCs and antibiotic resistance patterns observed among *S. aureus*, MRSA, and MDRSA carried by workers may represent the population of *S. aureus* circulating among hogs at the industrial hog operations where workers are employed, as has been observed elsewhere (Frana et al., 2013; Van Cleef et al., 2011). We did not sample animals from these farms, however, precluding confirmation of this hypothesis.

Livestock-associated MRSA was not a frequent nasal colonizer of participants in this study compared to individuals with intensive livestock exposure in other livestock-associated *S.*

aureus persistence studies (Graveland et al., 2011; Köck, Loth, et al., 2012; Verkade et al., 2013). Overall, the mean daily prevalence of *S. aureus* nasal carriage among participants in this study (65%) was much higher than typical population prevalence estimates of 20-40% (Wertheim et al., 2005), suggesting that individuals employed at industrial hog operations are exposed to a unique reservoir of *S. aureus* compared to the general population*,* and that carriage of livestock-associated *S. aureus* occurs in addition to carriage of human-adapted *S. aureus* strains, as has been suggested elsewhere (Verkade et al., 2013). Interestingly, livestock-associated *S. aureus* comprised the majority of *S. aureus* observed (mean daily prevalence: 51%). The relatively low mean daily prevalence of *scn*-positive *S. aureus* (14%) among participants in this study compared to the general population may indicate that livestock-associated *S. aureus* is competing with human-adapted strains to colonize the nasal passages of livestock workers in North Carolina (Verkade et al., 2013), or that this working population is less frequently colonized with human-adapted strains than the general population.

We used tetracycline resistance as a marker of livestock association in a previous study of carriage of *S. aureus* among livestock workers in North Carolina (Rinsky et al., 2013). We did not include tetracycline resistance as a formal marker in the present study because its capacity to distinguish human-adapted from livestock-associated *S. aureus* has only been described in the literature within the context of CC398 (Price et al., 2012), However, the tetracycline class of antibiotics is the most widely used in food animal production in the United States; 5.6 million kg of tetracycline were sold for domestic use in food animals in 2011 (Food and Drug Administration, 2011; Love et al., 2011). Consistent with knowledge about the use of tetracycline in the United States, we observed a high degree of overlap between tetracycline resistance and absence of *scn* (marker of livestock association used). All tetracycline-resistant isolates observed in this study (n=138) were *scn*-negative and most (80%) belonged to CC398, with the remaining belonging to CC9. Our findings support tetracycline resistance as a marker of livestock association when used among CC398 isolates. More extensive surveillance (including among animals) may be required to confirm whether tetracycline resistance is useful as an indicator of livestock association beyond the scope of CC398, such as within CC9. Continued efforts to identify and validate potential markers of livestock association will aid in improving the sensitivity and specificity of the definition of livestock association.

Our findings differ from previous work examining persistence of livestock-associated *S. aureus* among individuals exposed to industrial livestock production. First, we observed a relatively low prevalence of carriage of MRSA CC398 compared with previous studies of swine operation workers (Frana et al., 2013; Graveland et al., 2011; Köck, Loth, et al., 2012; Verkade et al., 2013). Second, we observed a relatively high prevalence of CC9, a clone that has been described in livestock and livestock workers in Asian countries (Cui et al., 2009; Garcia-Graells et al., 2012; Neela et al., 2009), but infrequently among livestock workers in the United States (Rinsky et al., 2013). Ninety-eight percent of CC9 isolates were *scn*-negative (53/54), and half were both *scn*-negative and tetracycline-resistant (27/54). Our findings suggest that CC9 may circulate within a livestock reservoir in the United States; however, further research including animal sampling is needed to confirm this hypothesis. Third, many studies examining persistence of livestock-associated *S. aureus* or livestock-associated MRSA have concluded that nasal carriage is an artifact of “contamination” from hand-to-nose contact or bioaerosols, and that carriage is transient after short periods away from the hog production environment (Frana et al., 2013; Graveland et al., 2011; Van Cleef et al., 2011). Recent studies of individuals with high frequency of exposure (e.g. livestock workers, farmers, veterinarians) found at least some reduction in prevalence of carriage following periods of low or no exposure as short as 24 hours (Graveland et al., 2011; Köck, Loth, et al., 2012). In the present study, we observed no change in nasal carriage status for the various *S. aureus* endpoints, including livestock-associated strains, following up to 96 hours away from work.

This lack of an association may indicate that time away from work is not related to loss of carriage; or, is due to study limitations. Specifically, the present study was small; including 22 individuals with 14-15 sampling points each (327 observations). Small numbers of individuals experienced changes in *S. aureus* carriage states during the study period, resulting in a sample size insufficient for repeated measures analysis. However, even within the small sample size we were able to observe patterns of persistent, intermittent, and non-carriers of *S. aureus*, and livestock-associated *S. aureus*, similar to reports from larger studies of individuals exposed to livestock (Graveland et al., 2011; Verkade et al., 2013), hospital in-patients, and the general population (Wertheim et al., 2005).

In addition to small sample size, we only assessed one *S. aureus* isolate per nasal swab for genotype, antibiotic resistance phenotype, and absence of *scn*. Assessment of only one isolate does not reflect the diversity of *S. aureus* that may occur within participants' noses (Cespedes et al., 2005) and may be responsible for some of our anomalous *S. aureus* outcomes (e.g. only one observation each of *scn*-negative CC20 and *scn*-positive CC9). In addition, the design of the study resulted in an up to eight day holding time between self-swabbing and laboratory analysis of nasal swabs. A *S. aureus* survival study revealed that this holding time may have resulted in false negative outcomes if swabs were inoculated with 10^2 colony forming units or fewer **(Appendix A)**. We allowed for one "false" negative in our definition of persistent carriers to reduce potential misclassification of carriage states based on holding time. Allowing for one false negative also accounted for potential error in self collection of nasal swabs, a method that has been previously used and validated (Gamblin et al., 2013; Gilbert et al., 2007; Köck, Loth, et al., 2012; Van Cleef et al., 2010).

Participants independently completed journal entries reporting daily work activities which were used as proxy measures of exposure to *S. aureus* in the industrial hog production environment. Ideally, we would have complemented this exposure information with samples from pigs and the barn environment in order to increase the certainty that *S. aureus* detected among participants was present in the livestock production environment. As the presence of *S. aureus*, and MRSA specifically, has been known to vary between herds and operations (Smith et al., 2009), it is possible that our assumption that these proxy measures were representative of exposure to *S. aureus* at work was incorrect. Additionally, because all participants in this study identified as Hispanic, it is theoretically possible that the *S. aureus* characteristics and strain types we detected among participants were unrelated to livestock exposure and were instead unique to this ethnic group; however, we observe no evidence in the literature to support this. Overall, misclassification of outcomes or exposures could have introduced bias to our results. However, no evidence exists to indicate that misclassification was differential with respect to outcome or exposure and consequently, it is most likely to have biased our results toward the null or reduced precision of estimates.

Differences between our findings and other studies could be related to several aspects of industrial swine production in North Carolina. Prior studies of persistence of livestock-associated *S. aureus* carriage included individuals with short-term exposure (Frana et al., 2013; Van Cleef et al., 2011), or extended time away from livestock or work (Graveland et al., 2011; Köck, Loth, et al., 2012). More than two days away from work was uncommon in our study population and when it occurred, it was unplanned. Workers reported that having 24 hours away from work more than 1-2 times/month was rare, precluding evaluation of the effects of longer times away from work in this population. Most pigs in North Carolina are owned by vertically-integrated companies that determine the animals' breed, feed, and antibiotic administration, and they are often transported between confinements dedicated to life stages of animal growth (e.g. farrowing, wean-to-feeder, feeder-to-finish), all factors that could affect animal carriage of *S. aureus*. Previous studies at industrial hog operations in North Carolina have been conducted at specific operations with permission from corporate producers (Aneja et al., 2008; Blunden et al., 2005; Schiffman et al., 2008). Our study was not conducted with corporate producers and therefore the generalizability of our findings is not restricted to the types of operations that agree to participate in research.

Further work is needed to improve the state of knowledge about the temporal dynamics of nasal carriage of livestock-associated *S. aureus*, including MRSA and MDRSA, among livestock workers in the United States. Our findings suggest that a study with a follow-up period longer than two weeks among a population of workers with greater variability in time away from work may be necessary to observe changes in nasal carriage of these endpoints. Examining the temporal dynamics of nasal carriage among a larger cohort is also necessary to evaluate measures of association between carriage states and personal and work-related characteristics or activities. Additionally, in order to properly evaluate the magnitude and severity of the public health risk posed by livestock-associated *S. aureus*, livestock-associated MRSA, and livestock-associated MDRSA, research is needed to determine whether there is an association between persistence of nasal carriage of these bacteria and the occurrence of infections among industrial hog operation workers and their household and community contacts.

TABLES

Table 2.1. Distribution of characteristics among 22 industrial hog operation workers, North Carolina.

	N=22[a]	%
Personal Characteristics		
Age		
< 24	2	9.1
25-34	5	22.7
35-44	11	50
≥ 45	4	18.2
Male	12	54.6
Education		
< High School	6	27.3
≥ High School	15	68.2
Number of household members		
< 3	2	9.1
3-5	13	59.1
≥ 6	6	27.3
Children < 6 years old living in household	10	45.5
Pets inside home	6	27.3
Lives on same property as hog operation	5	22.7
Contact sports		
≥ 1 month ago	10	45.5
< 1 month ago	6	27.3
Work Characteristics[b]		
Years employed at current hog operation		
< 1	5	22.7
1-5	7	31.8
6-9	3	13.6
≥ 10	4	18.2
Average hours/week		
≤ 40	3	13.6
41-50	6	27.3
51-60	11	50
> 60	2	9.1
Life stage of hogs in contact with at work[c]		
Sows/farrow piglets/wean	20	90.9
Feeder/finish	7	31.8
Average number of hogs worked with per day[d]		
≤ 1,000	3	13.6
1,001- 5,000	8	22.7
> 5,000	5	36.4

[a] Totals for each characteristic may not sum to the total number of participants due to missing information.

[b] Reported at baseline.
[c] Totals do not sum to 22 because some participants had contact with pigs in multiple life stages
[d] Calculated by multiplying the average number of animals per barn at operation of employment by the number of barns worked in on an average day

Table 2.2. Overall occurrence of nasal carriage, mean daily prevalence, and carriage states with various *S. aureus*-related outcomes among 22 industrial hog operation workers, North Carolina.

			Carriage States, N (%)		
Outcome	Ever Carriage N[c] (%)	Mean Daily Prevalence, % (range)	Persistent	Intermittent	Non-carrier
S. aureus	19 (86.4)	65.3 (47.4 – 72.7)	12 (54.5)	7 (31.8)	3 (13.6)
scn-negative *S. aureus*[a]	16 (72.7)	50.6 (36.8 – 56.8)	10 (45.5)	6 (27.3)	6 (27.3)
scn-positive *S. aureus*	6 (27.3)	14.7 (10.5 – 18.2)	2 (9.1)	4 (18.2)	16 (72.7)
MRSA[b]	1 (4.5)	4.0 (0.0 – 4.5)	1 (4.5)	0.0	21 (95.5)
MDRSA	10 (45.5)	32.7 (21.1 – 36.4)	6 (27.3)	4 (18.2)	12 (54.5)
scn-negative MDRSA[a]	9 (40.9)	31.6 (21.1 – 36.4)	6 (27.3)	3 (13.6)	13 (59.1)
scn-positive MDRSA	1 (4.5)	1.1 (0 – 2.3)	0.0	1 (4.5)	21 (95.5)

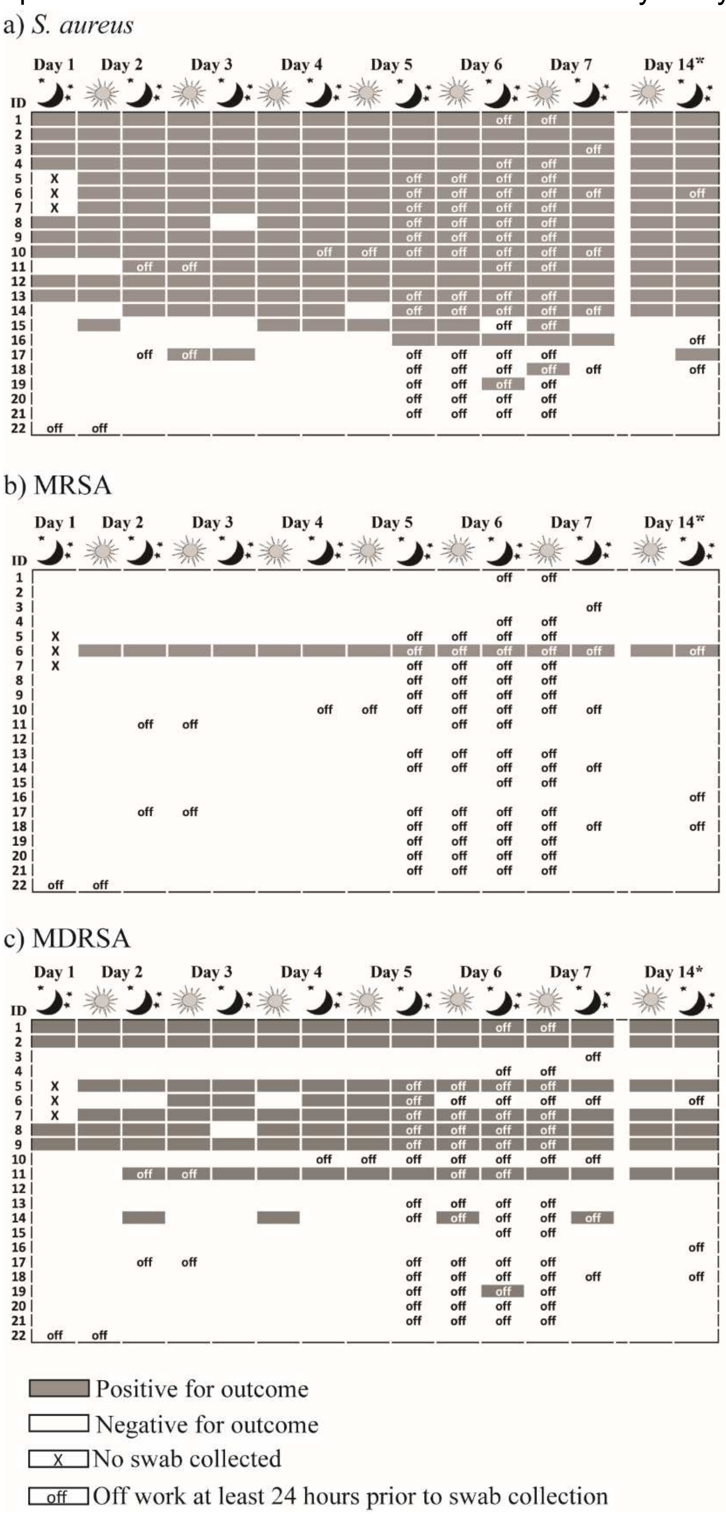
[a] *scn*-negative isolates were considered to be livestock-associated.

[b] All MRSA isolates were *scn*-negative

[c] It was possible for participants to carry both *scn*-positive and *scn*-negative *S. aureus* and MDRSA during the study period; therefore, the sum of these sub-classifications may not equal the total number of participants ever colonized by *S. aureus* and MDRSA.

FIGURES

Figure 2.1. Occurrence of (a) *S. aureus*, (b) MRSA, and (c) MDRSA among 22 industrial hog operation workers in North Carolina over a 14-day study period.



*Work status in the 24-hour period prior to collection of Day 14 AM swabs is unknown.

Figure 2.2. Distribution of clonal complex and tetracycline resistance among *S. aureus* isolated from 22 industrial hog operation workers in North Carolina over a 14-day study period, stratified by presence or absence of the *scn* gene.

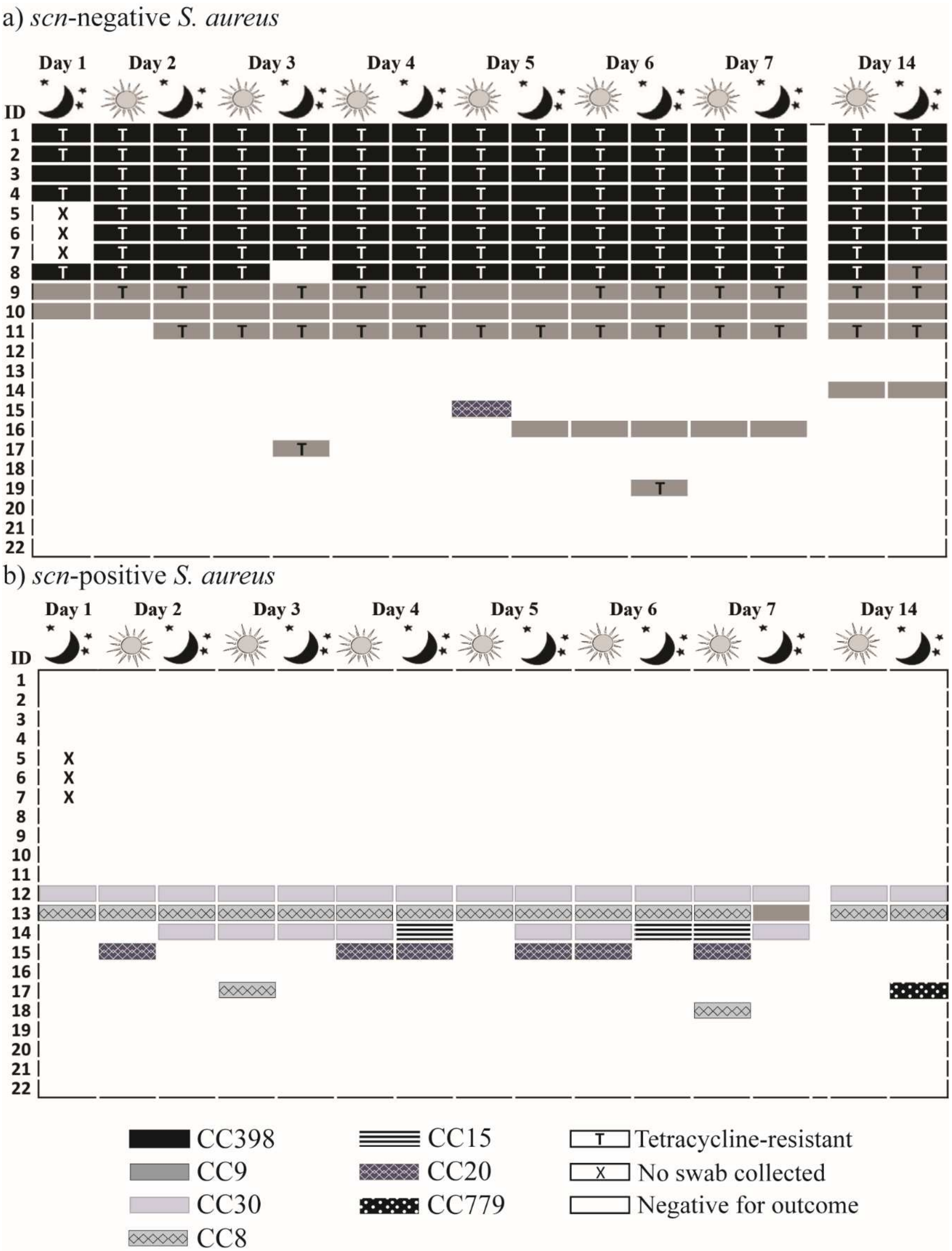
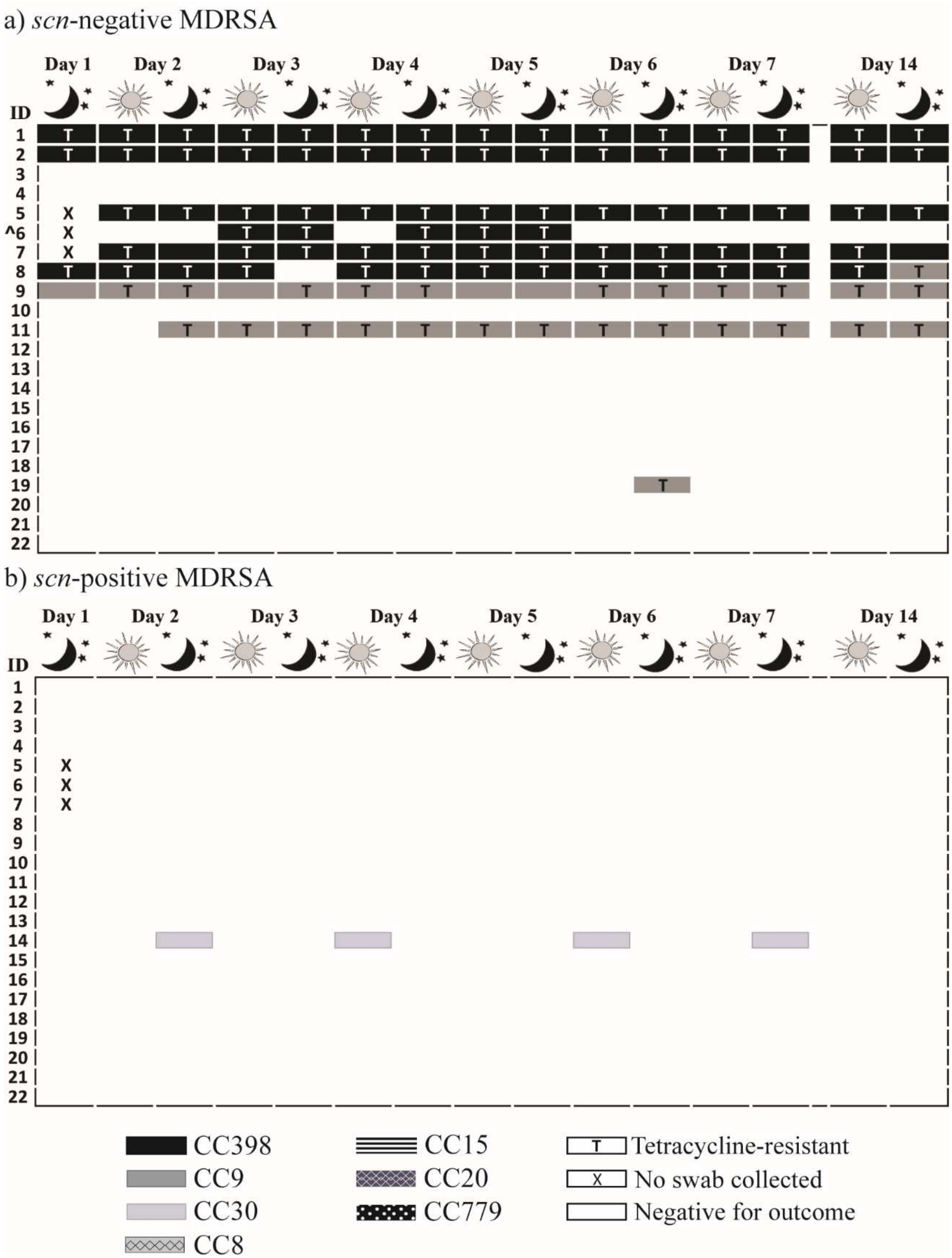


Figure 2.3. Distribution of clonal complex and tetracycline resistance among MDRSA isolated from 22 industrial hog operation workers in North Carolina over a 14-day study period, stratified by presence or absence of the *scn* gene.



^Isolates from Participant 6 were MRSA.

CHAPTER THREE: NASAL CARRIAGE OF LIVESTOCK-ASSOCIATED, ANTIBIOTIC-RESISTANT *STAPHYLOCOCCUS AUREUS* AND SYMPTOMS OF SKIN AND SOFT TISSUE INFECTION AMONG INDUSTRIAL HOG OPERATION WORKERS AND HOUSEHOLD CONTACTS IN NORTH CAROLINA

SUMMARY

Objective: This study aimed to assess *Staphylococcus aureus* nasal carriage and symptoms of recent skin and soft tissue infection (SSTI) among individuals directly and indirectly exposed to livestock-associated, antibiotic-resistant *S. aureus*, including methicillin- (MRSA) and multidrug-resistant *S. aureus* (MDRSA). Methods: Baseline data were collected from industrial hog operation workers and their household contacts via questionnaire between October 2013-February 2014 to assess risk factors for *S. aureus* nasal carriage and symptoms of SSTI in the prior three months. All participants also provided a nasal swab that was analyzed for *S. aureus*, including presence of *mec*A (indicating MRSA), resistance to ≥3 classes of antibiotics (indicating MDRSA), absence of *scn* (indicating livestock association), and *spa* type. Results: Six of 103 industrial hog operation workers, 6 of 54 minor household members and none of 26 adult household members reported recent symptoms of SSTI. Overall, participants who carried *S. aureus* (PR: 4.5, 95% CI: 1.4, 14.9) or MDRSA (PR: 3.1, 95% CI: 1.2, 7.8) at baseline were more likely to report symptoms compared to those who did not have these bacteria in their noses. Among workers, MDRSA (PR: 6.3, 95% CI: 1.5, 38.4) and *scn*-negative *S. aureus* nasal carriers (PR: 5.1, 95% CI: 1.2, 22.2) were more likely to report recent symptoms. Workers who never wore a mask at work carried between 0.01 – 0.5 more $\log_{10}$CFUs of s*cn*-negative *S. aureus* in their noses than workers who only sometimes wore a mask. Discussion: This study is the first in the United States to describe self-reported symptoms of SSTI among industrial hog operation workers. Despite small numbers, our findings add to the growing body of work

suggesting individuals exposed to livestock-associated, antibiotic-resistant *S. aureus*, MRSA, and MDRSA in the United States may be at risk for developing skin and soft tissue infections.

INTRODUCTION

Over the past decade, strains of antibiotic-resistant *Staphylococcus aureus* adapted to animals have emerged globally among livestock, poultry, and other domesticated animals, as well as among people in contact with these animals (Smith et al., 2011). These *S. aureus* strains, which include methicillin-resistant (MRSA) and multidrug-resistant *S. aureus* (MDRSA), are commonly referred to as “livestock-associated” *S. aureus*. Clonal complex (CC) 398 is the most widely described strain of livestock-associated *S. aureus,* although CC9 is increasingly being reported in several Asian countries (Patchanee et al., 2014; Wagenaar et al., 2009), as well as the southeastern United States (Nadimpalli et al., 2014b). In Europe, nasal carriage and infection with livestock-associated *S. aureus* have been reported among individuals with no clear contact with animals (Benito et al., 2014; Lekkerkerk et al., 2012), leading some to suggest that human-to-human transmission may now be occurring in the community (Lekkerkerk et al., 2012; Van Rijen et al., 2014).

Despite high prevalence of livestock-associated *S. aureus* nasal carriage among occupationally-exposed cohorts (Smith et al., 2011), it remains unclear whether nasal carriage of these bacteria is associated with infection. Nasal carriage of hospital and community-associated *S. aureus* is an established risk factor for infection in the clinical setting (Wertheim et al., 2005). Individuals carrying *S. aureus* are hypothesized to contaminate their hands by touching the nose or face before transferring these bacteria to open cuts or wounds (Wertheim et al., 2005). However, livestock-associated *S. aureus* differ from hospital and community-associated strains (Ballhausen et al., 2014). Most livestock-associated *S. aureus* isolates lack genetic factors commonly associated with human infections, including *pvl*, enterotoxin-producing genes, and the immune evasion complex (Argudín et al., 2011; Price et al., 2012). Their capacity for human-to-human transmission also appears to be lower than many widespread *S. aureus* clonal complexes (Bootsma et al., 2011; Hetem et al., 2013). Among infections that have occurred among healthy individuals, risk factors other than livestock exposure have not been identified (Benito et al., 2014; Smith & Wardyn, 2015). However, infections with livestock-associated *S. aureus* are on the rise in several countries (Köck et al., 2013; Statens Serum Institut, 2014), and have recently been reported for the first time among livestock workers in the United States (Wardyn et al., 2015). Identifying modifiable risk factors for these infections is important for protecting worker and community health.

In the present study, we assessed nasal carriage of livestock-associated *S. aureus* among industrial hog operation workers in North Carolina and their household members and associations with recent reports of skin and soft tissue infection (SSTI). Using data from the baseline study visit for a four month, repeated-measures study of *S. aureus* nasal carriage among this population, we examined: (a) baseline prevalence and distribution of *S. aureus* nasal carriage patterns; (b) associations between *S. aureus* nasal carriage patterns and work exposures, (c) associations between *S. aureus* nasal carriage patterns and reported symptoms of SSTI in the three months prior to the baseline visit, and (d) the distribution of reported symptoms of SSTI by specific work exposures.

MATERIALS AND METHODS

Baseline data were collected between October 2013 and February 2014 by community organizers from the Rural Empowerment Association for Community Help (REACH) with researchers from the Gillings School of Global Public Health at the University of North Carolina at Chapel Hill (UNC) and the Bloomberg School of Public Health at Johns Hopkins University (JHU).

Data Collection

All participants for this study were recruited by community organizers from REACH via a snowball sampling approach. Community organizers recruited livestock workers who fit the following inclusion criteria: worked at an industrial hog operation; resided in North Carolina;

could speak English or *Spa*nish, and were at least 18 years old. Up to three individuals living in the same household as a livestock worker were eligible to participate if they were at least seven years old and spoke English or *Spa*nish. Before participating, adult participants provided written informed consent. Written parental permission and informed assent were collected for participants seven to 17 years of age.

Participants were enrolled in multiple cycles. Upon enrollment, participants responded to a baseline questionnaire administered by a community organizer or researcher. The baseline questionnaire assessed demographic information, household member characteristics, work activities, risk factors for exposure to antibiotic-resistant *S. aureus*, and symptoms of SSTI in the previous three months. Participants also provided a self-collected BD BBL CultureSwab from both of their nares after being trained with study protocols. Every two weeks over the next four months, community organizers administered a bi-weekly follow-up questionnaire and supervised participants as they self-collected nasal swabs using the same swabbing protocol. Only baseline findings from this study will be discussed here.

Detection of *S. aureus* and MRSA

Baseline swabs were transported to UNC at 4°C within 12 hours of collection. A trip blank was included with each shipment to confirm lack of contamination during transport. Within three days of arrival, the end of each swab was removed and vigorously shaken in one ml of phosphate-buffered saline for 60 seconds. A portion of this eluate was plate spread on CHROMagar™ Staph aureus (BD, Franklin Lakes, NJ) (CSA) for the purpose of quantification, while the remaining eluate and swab were refrigerated at 4°C. After incubation at 37°C for 24 hours, colonies with the correct phenotype on CSA were hand counted and recorded as *S. aureus* colony forming units (CFU) per swab. If plating of the neat solution yielded colonies that were too numerous to count, serial dilutions from the remaining eluate were plate spread on CSA and incubated at 37°C for 24 hours. At least two colonies with morphology characteristic of *S. aureus* on CSA were streaked to isolation. Meanwhile, swabs negative for presumptive *S.*

aureus on CSA were enriched overnight at 37°C in 10 ml of Mueller-Hinton broth containing 6.5% NaCl. A loopful of Mueller-Hinton broth was streaked onto both Baird Parker and CSA plates to increase sensitivity of detection (Nadimpalli et al., 2013), and incubated at 37°C for 24 hours. Up to two colonies with morphology characteristic of *S. aureus* on either media were then streaked to isolation. In total, presumptive *S. aureus* colonies were isolated within two to four days from each nasal swab. Presumptive colonies were archived at -80°C in brain heart infusion broth with 15% (w/v) glycerol.

A crude DNA extraction was performed on each isolate using a protocol adapted from Reischl *et al*. (Reischl et al., 2000). Multiplex PCR was used to amplify the *spa*, *scn*, *mec*A, and *mec*C genes. Strain LGA251 (provided courtesy of Dr. Meghan Davis, JHU) was used as an extraction and PCR control for *spa* and *mec*C, while a clinical MRSA isolate (courtesy of Dr. Jill Stewart, UNC) was used as an extraction and PCR control for *spa*, *scn*, and *mec*A. Sterile water was used as a negative control. PCR products were visualized on 2% agarose gels stained with ethydium bromide. Colonies positive for *spa* were *S. aureus*; isolates positive for *spa* and either *mec*A or *mec*C were MRSA.

Singlepex PCR was used to evaluate the presence of *pvl* among all *S. aureus* isolates (Lina et al., 1999). A clinical isolate (courtesy of Dr. Jill Stewart, UNC) was used as a positive control and sterile water was used as a negative control. PCR products were visualized on 1.5% agarose gels stained with ethydium bromide.

Assessment of antibiotic susceptibility

One isolate from each *S. aureus*-positive nasal swab was assessed for susceptibility to 14 classes of antibiotics: aminoglycosides, β-lactams, cephalosporins, floroquinolones, glycopeptides, lincosamides, lipopeptides, macrolides, nitrofurans, oxazolidones, rifamycin, streptogramins, sulfonamide/methoprim, and tetracyclines (see **Appendix B: Table B1** for a listing of antibiotics used), using the Phoenix Automated Microbiology System (BD Diagnostic

Systems, *Spa*rks, MD). Testing was completed by the Clinical Microbiology Laboratory at the Johns Hopkins Hospital.

S. aureus isolates resistant to three or more classes of antibiotics were MDRSA.

Molecular analysis

The *spa* gene was amplified and sequenced for one isolate from each *S. aureus*-positive nasal swab using methods described previously (European Union Reference Laboratory for Antimicrobial Resistance, 2009). All isolates were characterized by *spa* typing using the Ridom StaphType software and the Ridom SpaServer (http://spa.ridom.de/index.shtml). We assigned clonal complexes (CCs) 398 or 9 to isolates with *spa* types that have previously been associated with these CCs in the literature.

Indicators of livestock association

There are currently no established markers for livestock-associated *S. aureus*. We examined four indicators of livestock association among *S. aureus* isolates: strain type CC398, strain type CC9, absence of *scn*, and tetracycline resistance. We have used CC398, absence of *scn*, and tetracycline resistance as indicators of livestock-association in previous work where rationale for these indicators is provided (Nadimpalli et al., 2014b; Rinsky et al., 2013). We examined CC9 as a possible indicator of livestock association in this study due to increased reports among livestock herds and people in contact with these herds in several Asian countries (Asai et al., 2012; Fang et al., 2014; Patchanee et al., 2014; Wagenaar et al., 2009), and frequent observation of this strain type among industrial livestock operation workers from the same geographic region in which we conducted the present study (Nadimpalli et al., 2014b; Rinsky et al., 2013). However, the specificity of CC9 as a marker of livestock association has not yet been demonstrated in the literature.

Definition of infection outcome

The main infection outcome variable, “any symptoms of *S. aureus* skin and soft tissue infection in the past three months,” was created and coded as “Yes”, “No”, or “missing” based on participants' responses to the baseline questionnaire. This variable was coded "Yes" for participants who reported "Yes, in the past three months" to any of the following: *S. aureus* infection; skin boil; pus-filled abscess; red, painful, swollen skin bump or "pimple"; or spider bite that was itchy; "No" for participants who reported "No" to all of the above; or, as "Missing." Participants were shown pictures of *S. aureus* skin infections with each of these presentations prior to answering this question. Hereafter, we will refer to this outcome as "symptoms of SSTI."

Statistical Analysis

We examined the distribution of personal characteristics (age, gender, education, use of antibiotics, participation in contact sports), household characteristics (number of household members, pets, location of home on a hog operation) and workers' occupational exposures (time at job, average hours worked/week, number and age of hogs in contact with at work) within the study population. We compared the distributions of potential demographic and environmental risk factors for nasal carriage of antibiotic-resistant *S. aureus* between workers and household members.

We calculated the prevalence of carriage of *S. aureus*, MRSA, MDRSA among workers and their household members. We also calculated the prevalence of *S. aureus,* MRSA, and MDRSA exhibiting one or more of the following: strain type CC398, strain type CC9, absence of *scn*, and tetracycline resistance. Where sample size allowed, we examined associations between demographic and environmental risk factors and carriage of *S. aureus and related outcomes*. We generated crude prevalence ratios (PR) comparing carriage of these outcomes among workers to carriage among household members along with 95% confidence intervals (CI), using log binomial models using generalized estimating equations with an exchangeable correlation matrix to account for the non-independence of observations within households (Royall, 1986). Results for adult and minor household members were pooled for these analyses. Demographic and environmental risk factors that were associated with both participant type (worker versus household member) and nasal carriage patterns were included in these models, where sample size allowed. However, adjusted PRs are not presented here because inclusion of these confounders did not change the strength or precision of effect estimates.

Among workers, we examined associations between occupational exposures and nasal carriage of MDRSA, *scn*-negative *S. aureus*, and tetracycline-resistant *S. aureus* using log binomial models. Crude prevalence ratios and 95% CIs were generated using generalized estimating equations with an exchangeable correlation matrix to account for the non-independence of observations within households. We used linear regression models to compare quantitative nasal carriage of MDRSA, *scn*-negative *S. aureus*, and tetracycline-resistant *S. aureus* among participants who reported specific occupational exposures compared to those who did not. We assumed that the genetic and phenotypic characteristics of one *S. aureus* colony from an individual were indicative of all *S. aureus* colonies from that individual in order to compute quantitative nasal carriage of MDRSA, *scn*-negative *S. aureus*, and tetracycline-resistant *S. aureus*. For *S. aureus*-positive individuals for whom we did not have a *S. aureus* CFU/nasal swab count because their swab was only positive after Mueller-Hinton broth enrichment, we imputed a value of five *S. aureus* CFU/nasal swab (half the limit of detection). For individuals who were *S. aureus*-negative even after enrichment, we imputed a value of one *S. aureus* CFU/nasal swab. We $\log_{10}$-transformed CFU/nasal swab counts prior to including them in linear regression models. Estimates for the linear term and 95% CIs were generated using generalized estimating equations with an exchangeable correlation matrix to account for the non-independence of observations within households.

We used log binomial models to examine associations between baseline *S. aureus* nasal carriage patterns and symptoms of SSTI in the past three months. Crude prevalence ratios and 95% CIs were generated using generalized estimating equations with an exchangeable correlation matrix to account for the non-independence of observations within households. Evaluation of environmental and occupational exposures as potential confounders of these associations was not possible due to small numbers.

We used linear regression models to compare quantitative nasal carriage of *S. aureus*, MDRSA, and *scn*-negative *S. aureus* among participants who reported symptoms of SSTI versus those who did not. Quantitative MDRSA, *scn*-negative *S. aureus*, and tetracycline-resistant *S. aureus* were estimated as described above. Estimates for the linear term and 95% CIs were generated using generalized estimating equations with an exchangeable correlation matrix to account for the non-independence of observations within households.

Among workers, we also examined the distribution of reported symptoms of recent SSTI by specific work exposures. Statistical analysis of associations between exposures and symptoms of SSTI were not possible due to small numbers.

All analyses were performed using SAS 9.3 (Cary, NC).

RESULTS

Participant and household characteristics

Participant characteristics are reported in **Table 3.1**. One hundred eighty-six participants comprising 103 workers (56%), 26 adult household members (14%), and 54 minors (30%) completed baseline questionnaires and provided baseline nasal swabs. All but two participants identified as Hispanic or African-American. Traditional risk factors for nasal carriage of antibiotic-resistant *S. aureus* were uncommon among workers and adults, including playing contact sports (12% and 0% reported this risk factor, respectively), using a gym or workout facility (10% and 4% reported this risk factor, respectively), or using antibiotics (8% and 12% reported this risk factor, respectively). Among minors, recently playing contact sports (36%) and using a gym or workout facility (55%) were more common, but using antibiotics was uncommon (6%).

In total, 81 households participated. Household characteristics are provided in **Appendix B: Table B2**. Most households comprised three to five individuals (53/73) and 45% reported having children under seven in the home (33/75). Half reported having no health insurance (47/81). Care from a private doctor was most common (42/81).

Appendix B: Table B3 describes occupational exposures among the 103 participating workers. Most reported working upwards of 40 hours per week (71/99); working between 51-60 hours per week was most common (40/99). Common work activities included handling dead pigs (79/100) and giving pigs shots (70/100). Almost all workers (96%) reported always wearing boots or other foot protection while at work, but always wearing gloves (86%), and always wearing long sleeves and pants or coveralls (86%) were slightly less common. Only 37% of workers reported always wearing a face mask at work.

Prevalence of *S. aureus*, MRSA, and MDRSA

S. aureus, MRSA, and MDRSA nasal carriage patterns, the distribution of each of these patterns by indicators of livestock association, and prevalence ratios comparing workers' nasal carriage to household members' nasal carriage are presented in **Table 3.2**.

Forty-five of 103 workers (44%) and 31 of 80 household members (39%) carried *S. aureus* at baseline. One worker was positive for MRSA. Twenty-one workers (20%) and eight household members (10%) carried MDRSA.

We observed elevated prevalence of MDRSA (PR: 2.0, 95% CI: 1.0, 4.0), *scn*-negative *S. aureus* 2.2 (1.4, 4.7), tetracycline-resistant *S. aureus* (PR: 3.0, 95% CI: 1.2, 7.8), tetracycline-resistant MDRSA (PR: 5.1, 95% CI: 1.2, 22.4), and *scn*-negative, tetracycline-resistant *S. aureus* (PR: 3.1, 95% CI: 1.1, 9.0) among workers compared to household members. *S. aureus* CC9 was only observed among workers (8/103). *S. aureus* CC398, including CC398 with additional indicators of livestock-association, was observed among both workers and household members.

We did not observe *pvl* among any *S. aureus* isolates in this study.

The distribution of *S. aureus* colony forming units (CFUs) recovered from *S. aureus*-positive nasal swabs is presented by participant type in **Appendix B: Figure B1**.

Antibiotic resistance patterns

Among the *S. aureus* isolates analyzed for this study, complete or intermediate resistance was observed to all 14 of the antibiotic classes tested except nitrofurans, glycopeptides, oxazolidones, rifamycin, and sulfonamide/methoprim.

Antibiotic resistance patterns for *S. aureus* isolates recovered from participants in this study are described in **Appendix B: Table B4**. *S. aureus* carried by workers exhibited a larger diversity of antibiotic-resistance patterns than *S. aureus* carried by household members.

Distribution of *spa* types and evidence of household transmission

Appendix B: Table B5 depicts the frequency and distribution of *S. aureus spa* types observed among workers, adults, and minors.

Ten households contained at least two individuals who carried the same *S. aureus spa* type at baseline. Eight of these ten were worker-household member pairs, one of ten was a household member-household member pair, and one of ten was a worker-worker pair. Only non-CC398 worker-household member pairs were observed (t645, t7226, t233, t659, t4976, t701, t094), although one pair carried tetracycline-resistant *S. aureus* (t701). One of ten households contained two minors carrying the same *S. aureus spa* type (t5739/CC398), and one of ten households contained two workers carrying the same *S. aureus spa* type (t337/CC9, both *scn*-negative).

Occupational and environmental risk factors for nasal carriage

Associations between specific work activities and nasal carriage of MDRSA, *scn*-negative *S. aureus*, and tetracycline-resistant *S. aureus* are presented in **Table 3.3**. The unadjusted prevalence of MDRSA (PR: 2.6, 95% CI: 2.1, 3.3) and tetracycline-resistant *S. aureus* (PR: 3.0, 95% CI: 2.2, 4.0) increased for every 1000 additional weaned pigs with whom workers reported direct contact. MDRSA (PR: 3.9, 95% CI: 1.0, 15.2) and s*cn*-negative *S. aureus* was more common among workers who reported never wearing a mask versus those who always wore a mask at work (PR: 5.0, 95% CI: 1.2, 21.4), We observed some differences in the amount of *S. aureus* log CFUs in the noses of participants who worked with weaned pigs, never wore a mask at work, and administered shots versus those who were not exposed to these activities. Workers' exposure to 1000 additional weaned pigs resulted in nasal carriage of slightly more $\log_{10}$CFUs of MDRSA (B-estimate: 1.1, 95% CI: 0.1 – 2.1) and tetracycline-resistant *S. aureus* (B-estimate: 1.1, 95% CI: 0.2 – 2.1) than workers who were positive for these nasal outcomes but not exposed to weaned pigs. Workers who administered shots to pigs carried between 0.2 – 0.8 more $\log_{10}$ CFUs of tetracycline-resistant *S. aureus* (B-estimate: 0.5, 95% CI: 0.2, 0.8) and between 0.1 – 0.7 more $\log_{10}$CFUs of s*cn*-negative *S. aureus* (B-estimate: 0.4, 95% CI: 0.1, 0.7) than workers who were positive for these outcomes but did not administer shots. Workers who never wore a mask at work carried between 0.01 – 0.5 more $\log_{10}$CFUs of s*cn*-negative *S. aureus* than workers who only sometimes wore a mask, but were positive for s*cn*-negative *S. aureus* (B-estimate: 0.3, 95% CI: 0.01, 0.5).

We did not observe any associations between participant and household characteristics reported in Tables **3.1** and **3.2** and *S. aureus* nasal carriage patterns (data not shown). Examination of these associations was not possible for some *S. aureus* nasal carriage patterns (e.g. MRSA) due to small numbers.

Prevalence of and risk factors for reported symptoms of SSTI

Six of 103 workers (5.8%) and six of 80 household members (7.5%) reported symptoms of SSTI in the three months prior to the baseline visit. Among the six workers reporting symptoms, three reported a swollen skin bump or "pimple," two reported a skin boil, and one reported a spider bite that was itchy. One of the workers with a swollen skin bump reported this symptom was doctor-diagnosed as a *S. aureus* infection. All household members who reported symptoms were minors. Among the six minors reporting symptoms, three reported a swollen skin bump or "pimple," and two reported a spider bite that was itchy. One minor reported a doctor-diagnosed *S. aureus* infection in the past three months, but did not describe its presentation. The worker and minor who reported doctor-diagnosed *S. aureus* infections lived in the same household. Another worker and minor who reported symptoms of infection lived with one another.

Associations between baseline *S. aureus* nasal carriage patterns and reported symptoms of SSTI during the past three months are presented in **Table 3.4**. Among all participants, we observed a higher unadjusted prevalence of SSTI symptoms among individuals who carried *S. aureus* (PR: 4.5, 95% CI: 1.4, 14.9) and MDRSA (PR: 3.1, 95% CI: 1.2, 7.8) at baseline. We did not observe any statistical difference in the quantity of *S. aureus* or MDRSA carried in the noses of participants who reported recent symptoms compared to those who were also positive for these outcomes, but did not report recent symptoms (data not shown). Among workers, we observed a higher crude prevalence of SSTI symptoms among individuals carrying MDRSA (PR: 8.8, 95% CI: 1.8, 43.9) and *scn*-negative *S. aureus* (PR: 5.1, 95% CI: 1.2, 22.2) at baseline. We did not observe any statistical difference in the quantity of MDRSA or *scn*-negative *S. aureus* carried in the noses of workers who reported recent symptoms compared to those who were also positive for these outcomes, but did not report recent symptoms (data not shown). None of the six workers reporting recent symptoms carried *S. aureus* CC398 at baseline, but two carried *scn*-negative *S. aureus* CC9, one carried *scn*-negative, tetracycline-resistant CC5, and one carried tetracycline-resistant t4976 (data not shown). *S. aureus* t4976 was also carried by a minor in this worker's household, although tetracycline-susceptible; this minor also reported symptoms of infection in the past three months.

The distribution of reported symptoms of SSTI by workers' occupational exposures is provided in **Appendix B: Table B3.** All six workers who reported symptoms of recent SSTI also reported administering shots to hogs at work and eating at the hog operation. Five of six workers reported handling dead hogs at work and five of six reported working with breeding sows and young pigs (sows, farrow piglets, wean, and/or nursery). None reported always wearing mask. On average, workers who reported symptoms reported working 52 hours per week (range: 48 – 56 hours/week). Examination of statistical associations between exposures and symptoms of SSTI were not possible due to small numbers.

DISCUSSION

In this baseline assessment of industrial hog operation workers and their household members in North Carolina, we observed recent symptoms of skin and soft tissue infection among workers and household members, some of whom carried livestock-associated, antibiotic-resistant *S. aureus* in their noses. Twelve participants, comprising six workers and six minors, reported symptoms of infection in the three months prior to enrollment. Participants who reported symptoms included two worker-minor pairs; one pair reported their symptoms were diagnosed as *S. aureus* infections. Symptoms of infection were more common among individuals who carried *S. aureus* or MDRSA at baseline. Among workers specifically, symptoms of infection were more common among individuals who carried MDRSA or *scn*-negative *S. aureus* at baseline. Despite small numbers, this is one of the first reports in the United States of skin and soft tissue infection among individuals with frequent and intensive exposure to industrial hog production, as well as their household members.

The incidence of *S. aureus* skin and soft tissue infection among the general U.S. population is not routinely surveyed. A 2013 study of patients attending a Pennsylvania clinic from 2001-2009 found the mean incidence rate of doctor-confirmed skin and soft tissue infections to be 3.3 cases/1,000 person-months (Casey, Cosgrove, et al., 2013). Symptoms of skin and soft tissue infection among industrial hog farm operators have recently been described by Wardyn *et al*. at twice this rate (6.6 cases/1,000 person-months) (Wardyn et al., 2015). The present study differed from the Wardyn *et al*. study in several ways. First, Wardyn *et al*. examined farm owners and operators, for whom exposure to animals and the barn environment is typically much less intensive than for livestock workers. Second, Wardyn *et al*. confirmed *S. aureus* infections using prospectively-collected skin swabs, while we assessed infection through participants' retrospective reports of symptoms. We did not employ a more intrusive assessment of infection (e.g. swabbing of ongoing infection sites), as workers' suspicions of reporting ongoing infections to academic researchers and fears about having infection outcomes reported to immigration authorities or employers could have precluded worker participation in this study (Mobed et al., 1992). However, this method of assessment may have incorrectly estimated the prevalence of SSTI among this population, as participants may have recalled SSTI symptoms that occurred earlier than the three months prior to the baseline questionnaire, reported skin afflictions that were not related to bacterial infection, or reported infections with an etiology different from *S. aureus*. We showed participants several pictures of serious *S. aureus* infections prior to asking them to report symptoms in order to minimize this possibility. The results presented here provide some of the first insights into the occurrence of skin and soft tissue infections among individuals directly and indirectly exposed to antibiotic-resistant *S. aureus* from the livestock farm environment in the United States.

To date, most livestock-associated *S. aureus* infections have presented as skin and soft tissue infections (Smith et al., 2015). Infections more severe than SSTI (e.g. bacteremia, pneumonia, and osteomyelitis, among others) have primarily been reported among elderly or immunocompromised individuals (Smith et al., 2015), who were infrequently represented in our study population (only one participant was over the age of 65, data not shown). The majority of livestock-associated *S. aureus* infections have been reported in Europe, where many countries have established surveillance systems for *S. aureus* infection that include molecular strain typing and assays for other indicators of livestock association (Smith et al., 2015). Because we asked participants to report symptoms of prior (and not current) infection, we were unable to evaluate the molecular and phenotypic characteristics of the *S. aureus* potentially responsible for these infections. Thus, we cannot determine whether these infections were caused by *S. aureus* with indicators of livestock association (CC398, CC9, tetracycline-resistance, or absence of *scn*). However, risk factors for community-associated and health care-associated antibiotic-resistant *S. aureus* infection (including recent use of antibiotics, playing contact sports, and recent use of a gym) were uncommon among workers. Some risk factors were more common among minors (playing contact sports, and recent use of a gym).

We evaluated *S. aureus* nasal carriage as a continuous rather than binary outcome in this study. Most studies of *S. aureus* nasal carriage among occupationally-exposed populations have evaluated *S. aureus*, MRSA, and/or MDRSA as present versus absent (Smith et al., 2011). Evaluation of *S. aureus* as a binary outcome is common in the clinical setting, where a quantitative understanding of *S. aureus* in the nose or at an infection site does not influence treatment. However, from an epidemiological perspective, evaluation of *S. aureus* nasal carriage as a continuous outcome affords increased statistical power to identify risk factors for nasal carriage and associations with disease outcomes. In the present study, we observed that workers who reported administering shots to pigs, working with weaned pigs, and never wearing a mask carried more *S. aureus*, MDRSA, tetracycline-resistant *S. aureus,* and/or *scn*-negative *S. aureus* in their noses than workers who did not report these exposures but were also positive for these outcomes. These data suggest that administering shots to pigs and working with weaned pigs may result in increased exposure to these bacteria, resulting in an elevated nasal dose. Meanwhile, using a face mask may reduce the dose of *S. aureus* that enters the nares. Evaluating *S. aureus* nasal carriage as a continuous outcome in future studies may provide additional evidence for these associations.

As with previous studies we have conducted in this region of the United States (Nadimpalli et al., 2014b; Rinsky et al., 2013), industrial hog operation workers and household members were recruited via snowball sampling, rather than via random sampling from an enumerated population (e.g. employee records). Thus, it is unclear whether our findings are generalizable to all livestock workers in the United States. Additionally, most workers in this study identified as Hispanic (89%). Theoretically, it is possible that the *S. aureus* characteristics and strain types we detected among workers in this study were unrelated to livestock exposure and were instead unique to this ethnic group; however, we observe no evidence in the literature to support this. Overall, the potential for exposure to and infection with antibiotic-resistant *S. aureus* among the estimated 292,000 livestock workers employed in the United States in 2012

(Census of Agriculture, 2007; United States Department of Agriculture, 2010; United States Department of Commerce, 2010) merits investigation. This population is often difficult to engage for academic researchers.

Additional research is needed to establish the direction and temporality of the association between nasal carriage of livestock-associated *S. aureus* and skin and soft tissue infection. Despite small numbers, we observed that nasal carriage of *S. aureus,* MDRSA, and *scn*-negative *S. aureus* was more common among individuals who reported recent symptoms of skin and soft tissue infection. Because we examined cross-sectional data, we cannot determine whether nasal carriage with these bacteria preceded or succeeded symptoms of infection. Still, this association is in accordance with previously observed associations between *S. aureus* nasal carriage and infection in the hospital setting (Wertheim et al., 2005). Interestingly, we also observed that using a face mask at work may reduce the dose of MDRSA and *scn*-negative *S. aureus* to workers' noses. None of the workers who reported always wearing a face mask reported recent symptoms of skin and soft tissue infection; however, only a small number of workers reported such symptoms. Overall, this work adds to the growing body of work suggesting individuals exposed to livestock-associated, antibiotic-resistant *S. aureus*, MRSA, and MDRSA in the United States may be at risk for developing skin and soft tissue infections.

TABLES

Table 3.1. Baseline characteristics of 183 industrial hog operation workers and household members participating in a cohort study of *S. aureus* nasal carriage in North Carolina, 2013-2014.

	Overall	Workers	Adults	Children
	N (%)[a]	N (%)[a]	N (%)[a]	N (%)[a]
Participants	183 (100)	103 (56)	26 (14)	54 (30)
Age in years, mean (SD)	30 (16)	39 (11)	38 (15)	11 (3)
Female	94 (52)	47 (46)	19 (73)	28 (53)
Race/ethnicity				
Hispanic	161 (89)	89 (89)	23 (88)	49 (92)
African-American	17 (9)	12 (12)	2 (8)	3 (6)
Caucasian	2 (1)	0 (0)	1 (4)	1 (2)
Education[b]				
K-5th grade	-	-	-	28 (54)
6-8th grade	-	-	-	17 (33)
9-11th grade	-	-	-	7 (13)
<High school[b]	107 (60)	48 (48)	7 (27)	-
≥High school[b]	72 (40)	53 (52)	19 (73)	-
Contact sports				
≤3 month ago	31 (17)	12 (12)	0 (0)	19 (36)
>3 month ago	151 (83)	91 (88)	26 (100)	34 (64)
Missing	1	0	0	1
Used gym or workout facility				
≤3 month ago	40 (22)	10 (10)	1 (4)	29 (55)
>3 month ago	142 (78)	93 (90)	25 (96)	24 (45)
Missing	1	0	0	1
Used antibiotics				
≤3 month ago	13 (7)	8 (8)	3 (12)	3 (6)
>3 month ago	165 (93)	91 (92)	23 (88)	51 (94)
Missing	5	4	0	1

[a]Totals for each characteristic may not sum to the total number of participants due to missing information.

[b]"Overall" column includes results from minors.

Table 3.2. Prevalence and distribution of *S. aureus* nasal carriage patterns among 183 industrial hog operation workers and household members.

	Overall	Workers	Household Members	PR (95% CI)[a]
	N (%)	N (%)	N (%)	
Participants	183 (100)	103 (100)	80 (100)	
S. aureus	76 (42)	45 (44)	31 (39)	1.1 (0.8, 1.5)
MRSA	1 (1)	1 (1)	0	--
MDRSA	29 (16)	21 (20)	8 (10)	2.0 (1.0, 4.0)
scn-negative				
S. aureus	25 (14)	20 (19)	5 (6)	2.9 (1.3, 6.5)
MRSA	1 (1)	1 (1)	0	--
MDRSA	18 (10)	14 (14)	4 (5)	2.6 (1.0, 6.7)
tetracycline-resistant				
S. aureus	21 (12)	17 (17)	4 (5)	3.3 (1.3, 8.5)
MRSA	1 (1)	1 (1)	0 (0)	--
MDRSA	15 (8)	13 (13)	2 (3)	5.2 (1.1, 23.8)
CC398[b]				
S. aureus	10 (6)	6 (6)	4 (5)	1.2 (0.3, 4.2)
MRSA	1 (1)	1 (1)	0 (0)	--
MDRSA	8 (4)	6 (6)	2 (3)	2.0 (0.5, 12.0)
CC9[c]				
S. aureus	8 (4)	8 (8)	0	--
MRSA	0	0	0	--
MDRSA	6 (3)	6 (6)	0	--
scn-negative, tetracycline-resistant				
S. aureus	16 (9)	13 (13)	3 (4)	3.3 (1.1, 10.3)
MRSA	1 (1)	1 (1)	0	--
MDRSA	13 (7)	11 (11)	2 (3)	4.4 (1.0, 20.3)

[a]"Household Members" is referent category.
[b]All MRSA CC398 and MDRSA CC398 isolates were tetracycline-resistant and *scn*-negative. All *S. aureus* CC398 isolates from workers were tetracycline-resistant and *scn*-negative. 3/4 *S. aureus* CC398 isolates from household members were tetracycline-resistant and *scn*-negative.
[c]All CC9 isolates were *scn*-negative. 4/8 *S. aureus* CC9 were tetracycline-resistant. 3/6 MDRSA CC9 were tetracycline-resistant.

Table 3.3. Associations between reported work activities and nasal carriage of MDRSA, *scn*-negative *S. aureus, and* tetracycline-resistant *S. aureus* among industrial hog operation workers in North Carolina, 2013-2014.

		MDRSA			*scn*-negative *S. aureus*			tetracycline-resistant *S. aureus*		
	N	N	PR (95% CI)[a]	β (95% CI)[b]	N	PR (95% CI)[a]	β (95% CI)[b]	N	PR (95% CI)[a]	β (95% CI)[b]
Average number of wean pigs in direct contact with (in increments of 1000)	99	‘3	2.6 (2.1, 3.3)	1.1 (0.1,2.1)	2	0.9 (0.2, 3.6)	0.2 (-0.7, 1.2)	3	2.7 (2.2, 4.0)	1.1 (0.2, 2.1)
Frequency of mask use										
Never	18	6	3.9 (1.0, 15.2)	0.9 (-0.6, 2.4)	4	5.0 (1.2, 21.4)	0.3 (0, 0.5)	4	4.1 (0.9, 17.7)	0.2 (-0.1, 0.5)
Sometimes	45	11	0.7 (0.3, 1.8)		11	1.6 (0.5, 5.8)		9	0.9 (0.3, 2.5)	
Always	37	2	ref		3	ref		2	ref	
Gives shots to hogs										
Yes	70	17	3.9 (0.9, 16.9)	0.4 (0, 0.7)	16	3.5 (0.8, 14.5)	0.4 (0.1, 0.7)	14	5.6 (0.8, 40.0)	0.5 (0.2, 0.8)
No	30	2	ref		2	ref		1	ref	

[a]Estimated with a log binomial regression model using a generalized estimating equation with an exchangeable correlation matrix to account for the non-independence of observations within households. Nasal carriage was modeled as a binary outcome.
[b]Estimated with a linear regression model using a generalized estimating equation with an exchangeable correlation matrix to account for the non-independence of observations within households. Nasal carriage was modeled as a continuous outcome.

Table 3.4. Association of baseline *S. aureus* nasal carriage patterns with any symptoms of *S. aureus* infection during previous three months among industrial hog operation workers and their child household members, North Carolina, 2013-2014.

	Overall			Worker			Household Members[a]		
	Total N	Symptoms[b] N (%)	PR (95% CI)	Total N	Symptoms[b] (%)	PR (95% CI)	Total N	Symptoms[b] N (%)	PR (95% CI)
S. aureus									
Carrier	72	9 (13)	4.5 (1.4, 14.9)	42	5 (12)	6.8 (0.9, 53.0)	25	4 (16)	2.2 (0.4, 11.2)
Non-carrier	104	3 (3)	ref	57	1 (2)	ref	27	2 (7)	ref
MDRSA									
Carrier	25	4 (16)	3.1 (1.2, 7.8)	18	4 (22)	8.8 (1.8, 43.9)	5	0	---
Non-carrier	151	8 (5)	ref	81	2 (2)	ref	47	6 (13)	ref
scn-negative *S. aureus*									
Carrier	22	3 (14)	2.4 (0.5, 10.7)	17	3 (18)	5.1 (1.2, 22.2)	2	0	---
Non-carrier	154	9 (6)	ref	82	3 (4)	ref	50	6 (12)	ref
tetracycline-resistant *S. aureus*									
Carrier	18	2 (11)	1.6 (0.5, 4.9)	14	2 (14)	3.0 (0.6, 13.8)	1	0	---
Non-carrier	158	10 (6)	ref	85	4 (5)	ref	51	6 (12)	ref

[a]No adult household member reported symptoms of a *S. aureus* infection at baseline.

[b]Comprises individuals who reported "Yes, in the past three months" to any of the following: *S. aureus* infection ; skin boil; pus-filled abscess; red, painful, swollen skin bump or "pimple"; or spider bite that is itchy. Participants were shown pictures of *S. aureus* infections with each of these presentations prior to answering this question.

CHAPTER FOUR: DYNAMICS OF LIVESTOCK-ASSOCIATED, ANTIBIOTIC-RESISTANT *STAPHYLOCOCCUS AUREUS* NASAL CARRIAGE AMONG INDUSTRIAL HOG OPERATION WORKERS AND HOUSEHOLD CONTACTS IN NORTH CAROLINA OVER FOUR MONTHS

SUMMARY

Objective: This study aimed to evaluate the nasal carriage of livestock-associated, antibiotic-resistant *Staphylococcus aureus* among industrial hog operations workers and household contacts over four months in a region with a high density of intensive industrial animal production. Methods: Bi-weekly nasal swabs and questionnaire data were collected from industrial hog operation workers and their household contacts from October 2013-June 2014. Nasal swabs were analyzed for *S. aureus*, including presence of *mec*A (indicating MRSA), resistance to ≥3 classes of antibiotics (indicating MDRSA), absence of *scn* (indicating livestock association), and *spa* type. Results: We examined 1,433 nasal swabs from 98 industrial hog operation workers, 25 adult household members, and 52 minors. Thirty-three percent, 1%, and 13% of workers were persistent carriers of *S. aureus*, MRSA, and MDRSA, respectively, over the four month period; 32%, 0%, and 6% of household members persistently carried these respective outcomes. For most of the indicators of livestock association we examined, workers were more likely to be intermittent carriers and less likely to be non-carriers compared to household members. However, there was no difference in the distribution of *S. aureus* CC398 carriage between workers and household members (χ^2=1.11, p=0.57). CC9, a strain frequently detected among Asian hogs and hog farmers, was observed almost exclusively along workers. Frequency of household members' nasal carriage with livestock-associated *S. aureus* was affected by worker's frequency of nasal carriage with these outcomes, as well as whether or not workers reported using face masks. Discussion: We observed evidence that workers may be

directly or indirectly sharing livestock-associated, antibiotic-resistant *S. aureus* with their household contacts. Persistent or intermittent nasal carriage of *S. aureus* CC9 among almost 40% of workers suggests that the global epidemiology of livestock-associated *S. aureus* may be less region-specific than previously thought.

INTRODUCTION

Strains of antibiotic-resistant *Staphylococcus aureus*, including methicillin-resistant (MRSA) and multidrug-resistant *S. aureus* (MDRSA), are present among intensively-raised food animal reservoirs in the United States, Europe, and several Asian countries (Chuang et al., 2015; Smith et al., 2011). These bacteria can transmit from food animals to humans in direct contact with these animals, resulting in nasal colonization or infection (Benito et al., 2014; Wardyn et al., 2015). Genetic and phenotypic traits such as strain type, absence of the bacteriophage-encoded *scn* gene (Nadimpalli et al., 2014b), and tetracycline resistance (Benito et al., 2014) are commonly used to distinguish livestock-adapted *S. aureus* that may be colonizing or infecting humans from human-adapted *S. aureus*. Contact with pigs is the strongest risk factor for colonization and infection (Smith et al., 2011).

Recent studies have examined the persistence of livestock-adapted *S. aureus* nasal carriage among individuals who are frequently exposed to food animals, as well as their household members (Graveland et al., 2011; Verkade et al., 2014; Verkade et al., 2013). Persistent nasal carriage with human-adapted *S. aureus* is associated with higher bacterial loads, which increases the risk for dispersal to other humans and infection in the clinical setting (Wertheim et al., 2005). Studies in Europe have observed persistent nasal carriage with livestock-adapted *S. aureus* among farm workers and veterinarians, but there is some indication that prevalence decreases during periods of low to no exposure (Graveland et al., 2011). Persistence of livestock-adapted *S. aureus* among household members appears to be much less common and strongly linked to livestock exposure (Garcia-Graells et al., 2013; Graveland et al., 2011; Verkade et al., 2014). Thus far, there is limited evidence in Europe to support the hypothesis that persistence of nasal carriage with livestock-adapted *S. aureus* among occupationally-exposed cohorts is linked to transmission of these bacteria to household contacts or increased risk for infection.

The dynamics of livestock-adapted *S. aureus* nasal carriage among livestock workers and their household contacts may differ from Europe, particularly in regions where food animal production is concentrated, such as eastern North Carolina. Previous work suggests that the diversity of nasal carriage outcomes among workers and their household contacts and the intensity of workers' exposures are different in eastern North Carolina compared to Europe (Rinsky et al., 2013). First, clonal complex (CC) 9, a clone infrequently described outside of livestock and livestock workers in Asia (Chuang et al., 2015), appears to be relatively common among workers in this region (Nadimpalli et al., 2014b; Rinsky et al., 2013). Second, methicillin-susceptible *S. aureus* (MSSA) with characteristics of livestock association appears to predominate among workers and their household contacts instead of MRSA, as is common in many European countries (Nadimpalli et al., 2014b; Rinsky et al., 2013; Smith et al., 2011). Third, workers at industrial hog operations in parts of the United States report contact with livestock more than 50 hours per week, and days away from the food animal production environment are rare (Nadimpalli et al., 2014b). Last, workers' and household members' relative environmental exposure to industrial livestock operations may be unusually high compared to many agricultural regions in Europe. Eastern North Carolina contains the ten most hog-dense counties in the United States; there are between 2,500 - 3,500 hogs/km^2 in the top three of these counties (Feedstuffs, 24 May 2010). In addition to intensive hog production, intensive turkey production is also concentrated in this region (Webb, 2015). Overall, these differences could contribute to unique dynamics of livestock-adapted *S. aureus* nasal carriage among industrial hog operation workers and their household contacts in North Carolina, with implications for public health.

In the present study, we (a) examined persistence of nasal carriage with *S. aureus* and related outcomes among industrial hog operation workers and their household contacts during bi-weekly visits over four months; (b) assessed differences in bacterial loads between non-carriers, intermittent carriers, and persistent carriers of *S. aureus* and related outcomes; (c) examined whether typical personal and/or occupational exposures were associated with persistence of nasal carriage; (d) evaluated whether average within-worker changes in personal and occupational exposures the week prior to nasal sampling were associated with average within-worker changes in nasal carriage of *S. aureus* and related outcomes; and (e) assessed whether persistent *S. aureus* nasal carriage was associated with reported symptoms of skin and soft tissue infection among workers and their household contacts.

MATERIALS AND METHODS

Data were collected between October 2013 and June 2014 by community organizers from the Rural Empowerment Association for Community Help (REACH) with researchers from the Gillings School of Global Public Health at the University of North Carolina at Chapel Hill (UNC) and the Bloomberg School of Public Health at Johns Hopkins University (JHU).

Data Collection

All participants for this study were recruited by community organizers from REACH via a snowball sampling approach. Community organizers recruited livestock workers who fit the following inclusion criteria: worked at an industrial hog operation; resided in North Carolina; could speak English or Spanish, and were at least 18 years old. Up to three individuals living in the same household as a livestock worker were eligible to participate if they were at least seven years old and spoke English or Spanish. Before participating, adult participants provided written informed consent. Written parental permission and informed assent were collected for participants seven to 17 years of age.

Participants were enrolled in multiple cycles. Upon enrollment, participants responded to a baseline questionnaire administered by a community organizer or researcher. The baseline questionnaire assessed demographic information, household member characteristics, work activities, risk factors for exposure to antibiotic-resistant *S. aureus*, and symptoms of skin and soft tissue infection in the previous three months. Participants also provided a self-collected BD BBL CultureSwab™ (BD Diagnostics, Sparks, MD) from both of their nares after being trained with study protocols. Every two weeks over the next four months, community organizers administered a bi-weekly questionnaire that assessed recent personal and occupational exposures, injury, and symptoms of respiratory and skin infection. Community organizers also supervised participants as they self-collected bi-weekly nasal swabs using the same swabbing protocol.

Detection of *S. aureus* and MRSA

Swabs were stored at the REACH office at 4-8°C prior to transport to UNC, which typically occurred within six days of collection. Upon arrival, the end of each swab was removed using sterile methods and vigorously shaken in one ml of phosphate-buffered saline (PBS) for 60 seconds. Swabs and PBS eluates were either processed immediately or diluted with an equivalent volume of tryptic soy broth with 40% glycerol (w/v) and stored at -45°C for later processing. A portion of fresh or thawed PBS eluate was plate spread on CHROMagar™ Staph aureus (BD, Franklin Lakes, NJ) (CSA) for the purpose of quantification, while the remaining eluate and swab were refrigerated at 4°C. After incubation at 37°C for 24 hours, colonies with the correct phenotype on CSA were hand counted and recorded as *S. aureus* colony forming units (CFU) per swab. If plating of the neat solution yielded colonies that were too numerous to count, serial dilutions from the remaining eluate were plate spread on CSA and incubated at 37°C for 24 hours. At least two colonies with morphology characteristic of *S. aureus* on CSA were streaked to isolation. Meanwhile, swabs negative for presumptive *S. aureus* on CSA were enriched overnight at 37°C in 10 ml of Mueller-Hinton broth containing 6.5% NaCl. A loopful of Mueller-Hinton broth was streaked onto both Baird Parker and CSA plates to increase sensitivity of detection (Rinsky et al., 2013), and incubated at 37°C for 24 hours. Up to two colonies with morphology characteristic of *S. aureus* on either media were then streaked to isolation. Presumptive colonies were archived at -80°C in brain heart infusion broth with 15% (w/v) glycerol.

A crude DNA extraction was performed on each isolate using a protocol adapted from Reischl *et al*. (Reischl et al., 2000). Multiplex PCR was used to amplify the *spa*, *scn*, *mec*A, and *mec*C genes. LGA251 (provided courtesy of Dr. Meghan Davis, JHU) was used as an extraction and PCR control for spa and *mec*C, while a nasal colonization MRSA isolate (courtesy of Dr. Jill Stewart, UNC) was used as an extraction and PCR control for *spa*, *scn*, and *mec*A. Sterile water was used a negative control. PCR products were visualized on 2% agarose gels stained with ethydium bromide. Colonies positive for *spa* were *S. aureus*; isolates positive for *spa* and either *mec*A or *mec*C were MRSA.

Singlepex PCR was used to evaluate the presence of *pvl* among all *S. aureus* isolates (Lina et al., 1999). A nasal colonization isolate (courtesy of Dr. Jill Stewart, UNC) was used as a positive control and sterile water was used as a negative control. PCR products were visualized on 1.5% agarose gels stained with ethydium bromide.

Assessment of antibiotic susceptibility

One isolate from each *S. aureus*-positive nasal swab was assessed for susceptibility to 14 classes of antibiotics: aminoglycosides, β-lactams, cephalosporins, floroquinolones, glycopeptides, lincosamides, lipopeptides, macrolides, nitrofurans, oxazolidones, rifamycin, streptogramins, sulfonamide/methoprim, and tetracyclines (see **Appendix C: Table C1**) for a listing of antibiotics used), using the Phoenix Automated Microbiology System (BD Diagnostic Systems, Sparks, MD). Testing was completed by the Clinical Microbiology Laboratory at the Johns Hopkins Hospital.

S. aureus isolates resistant to three or more classes of antibiotics were MDRSA.

Molecular analysis

The *spa* gene was amplified and sequenced for one isolate from each *S. aureus*-positive nasal swab using methods described previously (European Union Reference Laboratory for Antimicrobial Resistance, 2009). All isolates were characterized by *spa* typing using the Ridom StaphType software and the Ridom SpaServer (http://spa.ridom.de/index.shtml). *spa* types were then assigned to putative clonal complexes (CCs) 398 or 9 (or neither) based on the existing literature.

Indicators of livestock association

There are currently no established markers for livestock-associated *S. aureus*. We examined four indicators of livestock association among *S. aureus* isolates: strain type CC398, strain type CC9, absence of *scn*, and tetracycline resistance. We have used CC398, absence of *scn*, and tetracycline resistance as indicators of livestock-association in previous work where rationale for these indicators is provided (Nadimpalli et al., 2014b; Rinsky et al., 2013). We examined CC9 as a possible indicator of livestock association in this study due to increased reports among livestock herds and people in contact with these herds in several Asian countries (Asai et al., 2012; Fang et al., 2014; Patchanee et al., 2014; Wagenaar et al., 2009), and frequent observation of this strain type among industrial livestock operation workers from the same geographic region in which we conducted the present study (Nadimpalli et al., 2014b; Rinsky et al., 2013). However, the specificity of CC9 as a marker of livestock association has not yet been demonstrated in the literature.

Statistical Analysis

We examined questionnaire and laboratory data from participants who completed at least six of eight bi-weekly follow-ups.

We examined whether sample collection and laboratory processing factors were associated with *S. aureus* recovery from participants' nasal swabs. We used linear and log binomial regression models to examine whether time-invariant sample collection factors (i.e. for some participants, community organizer who supervised self-collection of nasal swabs) were associated with binary or quantitative recovery of *S. aureus* and related outcomes from individuals’ nasal swabs. Estimates and 95% confidence intervals (CI) were generated using generalized estimating equations with an exchangeable correlation matrix to account for the non-independence of observations within subjects and within households. We used conditional fixed effects models to examine whether time-varying sample collection and sample storage factors (i.e. time of day when sample was collected, season when sample was collected, length of time between collection and processing, whether or not a swab was frozen prior to processing, and for some participants, community organizer who supervised self-collection of nasal swabs) were related to binary or quantitative recovery of *S. aureus* and related outcomes from individuals’ nasal swabs. Fixed effects models use repeated measures to make within-subject comparisons, and then average those differences across all individuals in a sample (Allison, 2005). Time-invariant participant characteristics that could influence the outcome are differenced out in these models; thus, each individual serves as his or her own control (Allison, 2005). Data affected by factors that were found to be significantly associated with *S. aureus* recovery were excluded from subsequent analyses.

For each participant, carrier indices for the following outcomes were calculated: *S. aureus*, MRSA, MDRSA, *scn*-negative *S. aureus*, tetracycline-resistant *S. aureus*, *S. aureus* CC398, and *S. aureus* CC9. Carrier indices were defined as the percent of nasal swabs contributed by an individual over the course of the study that were positive for *S. aureus* or a related outcome (each participant contributed up to nine nasal swabs). Individuals with a carrier index of 80% or higher for an outcome were defined as “persistent” carriers of that outcome; individuals with a carrier index greater than 0 but less than 80% for an outcome were defined as “intermittent” carriers of that outcome, and individuals with a carrier index of 0 were defined as “non-carriers” of that outcome (Van Belkum et al., 2009). Our definition of persistent carriage allowed for one negative swab in order to minimize misclassifications resulting from laboratory or sampling error (Van Belkum et al., 2009).

We examined the distributions of nasal carriage indices for *S. aureus*, MRSA, MDRSA, *scn*-negative *S. aureus*, tetracycline-resistant *S. aureus*, *S. aureus* CC398, and *S. aureus* CC9 among workers and their household contacts. We used the Rao-Scott chi-square test to test the hypothesis that the distributions of persistent, intermittent, and non-carriers of each these outcomes were similar between workers and household members, while accounting for within-household clustering. Using conditional log binomial regression models with robust standard errors to account for within-household clustering, we generated prevalence ratios (PR) and 95% CI comparing the prevalence of persistent carriers, intermittent carriers, and non-carriers of each outcome among workers to the prevalence of each carriage state among household members (referent group).

We used linear regression models to estimate the average quantity of *S. aureus* and related outcomes present in the noses of intermittent and persistent nasal carriers relative to non-carriers. We calculated quantitative *S. aureus*, MRSA, MDRSA, *scn*-negative *S. aureus*, tetracycline-resistant *S. aureus*, *S. aureus* CC398, *S. aureus* CC9 using the following method: for *S. aureus*-positive individuals for whom we obtained a *S. aureus* CFU/nasal swab count, we assumed that all colonies contributing to this count were *S. aureus*, MRSA, MDRSA, *scn*-negative *S. aureus*, tetracycline-resistant *S. aureus*, *S. aureus* CC398, and/or *S. aureus* CC9 based on PCR and antibiogram results for one colony from that individual. For *S. aureus*-positive individuals for whom we did not have a *S. aureus* CFU/nasal swab count because their swab was only positive after Mueller-Hinton broth enrichment, we imputed a value of five *S. aureus* CFU/nasal swab. For individuals who were *S. aureus*-negative even after enrichment, we imputed a value of one *S. aureus* CFU/nasal swab. We $\log_{10}$-transformed CFU/nasal swab counts prior to including them in linear regression models. Estimates for the linear term and 95% CIs were generated using generalized estimating equations with an exchangeable correlation matrix to account for the non-independence of observations from the same individual and within households.

We examined associations between potential predictors of persistent nasal carriage and nasal carriage indices among workers and among household contacts. Fixed or typical exposures that had been reported during the baseline visit (e.g. household size, number of pets in the household, whether or not workers in the household typically wore a face mask while at work) were evaluated as potential predictors of persistence using linear regression models. Prevalence ratios and 95% CIs were generated using generalized estimating equations with an exchangeable correlation matrix to account for the non-independence of observations within households.

Among workers, we used conditional fixed effects regression models to examine associations between workers' personal and occupational exposures and nasal carriage of *S. aureus* and related outcomes. We used conditional fixed effects logistic regression models to generate odds ratios (OR) and 95% CI that described associations between workers' personal and occupational exposures and the bi-weekly presence or absence of *S. aureus* and related outcomes in the nose. We used conditional fixed effects linear regression models to examine associations between workers' personal and occupational exposures and the amount of *S. aureus* and related outcomes recovered from nasal swabs ($\log_{10}$ CFUs).

Participants reported symptoms of recent skin and soft tissue infection during the baseline questionnaire; assessment and assignment of the outcome "any symptoms of *S. aureus* skin and soft tissue infection in the three months prior to the baseline visit" are described elsewhere (**Chapter 3**). Participants were asked to report some symptoms of skin and soft tissue infection during the bi-weekly questionnaire as part of a larger assessment of general symptoms of ill-health. We created the infection outcome variable "any symptoms of *S. aureus* skin and soft tissue infection in the past two weeks" based on participants' responses to bi-weekly questionnaires. This variable was coded "Yes" for participants if they reported "Yes, in the past two weeks” to any of the following: infected cut or skin injury; pus-filled abscess; red, painful, swollen skin bump or “pimple”; spider bite that is itchy, or skin rash. This variable was coded “No” for participants if they reported “No” to all of the above categories. Otherwise, this question was coded as missing. Hereafter, we will refer to this outcome as “symptoms of SSTI.”

We used log binomial models to examine associations between *S. aureus* nasal carriage states (persistent, intermittent, and non-carrier) among participants and a) symptoms of SSTI in the three months prior to enrollment as reported at baseline, and b) ever-reported symptoms of SSTI during the four month study. Crude prevalence ratios and 95% CIs were generated using generalized estimating equations with an exchangeable correlation matrix to account for the non-independence of observations within households. We were unable to evaluate associations between workers’ personal and occupational exposures and bi-weekly reports of this outcome due to small numbers. Stable associations between *S. aureus* nasal carriage indices among participants and ever-reported symptoms of SSTI during the four month study could not be generated due to small numbers. We were unable to use fixed effects models to evaluate associations between within-person changes in exposure and bi-weekly reports of this outcome due to small numbers.

We examined the distribution of *S. aureus spa* types that were observed during the same bi-weekly follow-up visit among workers and at least one other household contact. We refer to these *spa* types as concordant within-household *S. aureus spa* types.

All analyses were performed using SAS 9.4 (Cary, NC).

RESULTS

Study population

One hundred eighty-six participants comprising 103 workers (56%), 26 adult household members (14%), and 54 minors (30%) enrolled in this study. Participant characteristics, household-level characteristics, and workers’ typical occupational exposures are described elsewhere (**Chapter 3**).

A total of 1,588 nasal swabs were collected from participants between October 2013 and June 2014. We excluded from further analysis swabs that were frozen prior to processing (129/1588), as conditional fixed effects models revealed this factor to be strongly associated with a reduction in recovery of binary and quantitative *S. aureus* and related outcomes (data not shown). No other sample collection or laboratory processing factors (i.e. time of day when sample was collected, season when sample was collected, length of time between collection and processing, community organizer who supervised self-collection of nasal swabs) were observed to be associated with *S. aureus* recovery from participants' nasal swabs (data not shown). We also excluded from further analysis nasal swabs collected from participants who did not complete at least 6/8 bi-weekly visits (26/1588). We did not observe any differences in the distributions of age, sex, household size, or certain occupational exposures between workers who were excluded from analysis compared to those who completed at least 6/8 bi-weekly visits (data not shown). Too few adults and minors were excluded from analysis to examine statistical differences between these individuals and adults and minors who completed at least 6/8 bi-weekly visits. In total, 1,433 nasal swabs from 98 workers, 25 adult household members, and 52 minors were included in our analyses.

Persistence of nasal carriage with *S. aureus*, MRSA, MDRSA, and *S. aureus* with indicators of livestock association

The distribution of nasal carriage indices for *S. aureus*, MRSA, and MDRSA is presented by participant type in **Figure 4.1**. The prevalence of non-carriers, intermittent carriers, and persistent carriers of these outcomes among workers compared to household members is presented in **Appendix C: Table C1**.

Workers were less likely to be non-carriers of *S. aureus* over the four month period than household members (PR: 0.5, 95% CI: 0.3, 0.8), but there was no difference in the prevalence of intermittent or persistent *S. aureus* nasal carriage between these two groups. Similarly, workers were somewhat less likely to be non-carriers of MDRSA over the four month period than household members (PR: 0.8, 95% CI: 0.6, 1.0), but there was no difference in the prevalence of intermittent or persistent MDRSA nasal carriage between workers and household members.

MRSA was not frequently recovered from the noses of participants in this study. Ninety-five percent of workers and 96% of household members never carried MRSA throughout the four month study period. Only one worker persistently carried MRSA over four months; no household members were persistent MRSA carriers.

The distribution of nasal carriage indices for *S. aureus* with indicators of livestock association is presented by participant type in **Figure 4.2**. For three of the four indicators of livestock association we examined (absence of *scn*, tetracycline resistance, and CC9), workers were more likely to be intermittent carriers and less likely to be non-carriers of *S. aureus* with these indicators compared to household members. However, there was no difference in the distribution of non-carriers, intermittent carriers, and persistent carriers of *S. aureus* CC398 between workers and household members, even when comparing *S. aureus* CC398 with additional indicators of livestock association (absence of *scn*, tetracycline resistance) (**Figure 4.2** and **Appendix C: Table C1**).

S. aureus CC9 was observed intermittently among only two household members during the four month study, compared to 32 workers. Persistent *S. aureus* CC9 nasal carriage was observed exclusively among workers. This differed from the other indicators of livestock association we examined (absence of *scn*, tetracycline resistance, and CC398), as persistent nasal carriage with *S. aureus* exhibiting one or more of these other indicators was observed among both workers and household contacts.

We did not detect the *pvl* gene among any *S. aureus* isolates in this study.

Quantitative differences in *S. aureus* recovery by nasal carriage state

The average increase in *S. aureus* CFU recovered from persistent and intermittent nasal carriers relative to non-carriers is presented by *S. aureus*-related outcome in **Figure 4.3**.

For all outcomes we investigated except MRSA, persistent nasal carriers carried more *S. aureus* CFU relative to the null than intermittent carriers relative to the null (i.e., the confidence intervals for these estimates did not overlap).

The average MRSA CFU recovered from workers (β: 0.4, 95% CI: -0.2, 1.0) and household members (β: 0.8, 95% CI: -0.1, 1.7) who intermittently carried MRSA was not different from the null. However, relatively few workers (4/98) workers and household contacts (4/77) were intermittent MRSA nasal carriers over the four month study period. Only one worker persistently carried MRSA; the 95% CI for the β estimate comparing this individual's average MRSA CFU to the null overlaps with the 95% CI for the β estimate comparing the average MRSA CFU among household members who intermittently carried MRSA to the null. Because of small numbers, these estimates should be interpreted with caution.

Predictors of persistence of nasal carriage with *S. aureus* and related outcomes among workers and household contacts

Associations between occupational and personal exposures reported during the baseline visit that we considered to be time-stable (e.g. number of individuals living in a household, number of pets) and nasal carriage indices for *S. aureus* and related outcomes over four months are presented for workers and household members, respectively, in **Tables 4.1 and 4.2**.

Among workers, direct contact with every two additional pets was associated with a 0.3-7.7% increase in the percent of nasal swabs positive for MDRSA over four months, and a 2.0-7.8% increase in the percent of nasal swabs positive for *S. aureus* CC398. Living on a property where animals were raised was associated with a 0.1-27% increase in the percent of nasal swabs positive for *S. aureus* CC398 over four months. Living with every two additional household members was associated with a 0.01-15% increase in the percent of nasal swabs positive for *scn*-negative *S. aureus* and a 0.2-9.7% increase in the percent of nasal swabs positive for *S. aureus* CC9. Every 10% increase in the average *S. aureus* nasal carrier index among workers' household contacts was associated with a 3.5%-6.9% increase in the percent of workers' nasal swabs positive for *S. aureus*. Every ten additional years worked at any industrial hog operation over a worker's lifetime was associated with a 1.6-21% increase in the percent of nasal swabs positive for *scn*-negative *S. aureus.* Workers who reported never wearing a face mask at work had an additional 1.9-42% of nasal swabs positive for MDRSA and an additional 1.8-37% of nasal swabs positive for *scn*-negative *S. aureus* over four months, relative to workers who reported always wearing a face mask at work (**Table 4.1**).

Among household members, every 10% increase in the average *S. aureus* nasal carrier index among workers living in their household was associated with a 4.0%-8.6% increase in the percent of household members' nasal swabs positive for *S. aureus*. Every 10% increase in the average *scn*-negative *S. aureus* nasal carrier index among workers living in their household was associated with a 0.9-5.2% increase in the percent of household members' nasal swabs positive for *scn*-negative *S. aureus*. Living with at least one worker who reported never wearing a face mask at work was associated with a 2.2-35.4% increase in the percent of nasal swabs positive for *scn*-negative *S. aureus* and a 1.3-28% increase in the percent of nasal swabs positive for tetracycline-resistant *S. aureus*, compared to household members who lived with workers who reported always or sometimes wearing a face mask at work. Living with at least one worker who reported sometimes or never wearing a face mask at work was associated with a 1.7-18% increase in the percent of nasal swabs positive for *scn*-negative *S. aureus* and a 0.6-14.3% increase in the percent of nasal swabs positive for tetracycline-resistant *S. aureus*, compared to household members who lived with workers who reported always wearing a face mask at work (**Table 4.2**).

Within-worker effects of occupational exposures on within-worker nasal carriage of *S. aureus* and related outcomes

Results from conditional fixed effects models are provided in **Table 4.3**. Within workers, the odds of *S. aureus* CC9 nasal carriage was somewhat higher (OR: 1.5, 95% CI: 1.0, 2.2) with every ten additional hours worked at an industrial hog operation the week prior to nasal sampling. The odds of *S. aureus* nasal carriage remained unchanged, but the amount of *S. aureus* in workers' noses decreased when working in barns that were hot (β: -0.41, standard error (SE): 0.18), the week prior, relative to when barns were cool or comfortable. Only sometimes wearing a face mask at work (>0% but <80% of the time) the week prior to sampling was associated with increased odds of nasal carriage with MDRSA (OR: 4.7, 95% CI: 1.5, 15.4), tetracycline-resistant *S. aureus* (OR: 4.7, 1.5, 14.1), and *S. aureus* CC9 (OR: 3.9, 95% CI: 1.4, 10.8), compared to sampling points for which workers reported always wearing a mask (≥80% of the time) the week prior.

We did not observe that time away from the workplace was associated with within-worker changes in nasal carriage of *S. aureus* or related outcomes (data not shown). However, most workers' nasal swabs (678/790) were collected less than 24 hours since their last work shift.

Symptoms of skin and soft tissue infection and associations with *S. aureus* nasal carriage

Nine of 183 (5%) participants comprising seven workers, one adult, and one minor reported symptoms of SSTI during the four month study. Two workers reported an infected cut or skin injury, one worker reported a pus-filled abscess, two reported a red, painful, swollen skin bump or "pimple," four reported a spider bite, and three reported a skin rash. Three workers reported multiple symptoms of skin and soft tissue infection during the same study visit. One worker's rash reportedly persisted for two months. Five of seven workers who reported symptoms reported wearing a face mask less than 80% of the time on average during the four month study; one consistently reported never wearing a face mask (data not shown). One adult reported a skin rash and infected cut or skin injury during the same study visit. One minor reported a skin rash. Overall, the rate of reported symptoms of SSTI among industrial hog operation worker and their household contacts was 14.2 cases per 1,000 person-months. The incidence rate of reported symptoms of SSTI among workers specifically was 20.2 cases per

1,000 person-months. The incidence rate of reported symptoms of SSTI among household members specifically was 7.0 cases per 1,000 person-months.

Associations between nasal carriage indices for *S. aureus*-related outcomes and baseline reports of symptoms of SSTI are presented in **Table 4.4**. Overall, individuals with a higher *S. aureus* nasal carrier index (PR: 1.9, P5% CI: 1.1, 3.2) and a higher *S. aureus* CC9 nasal carrier index (PR: 2.1, 95% CI: 1.1, 3.8) were more likely to have reported symptoms of SSTI at the baseline visit. Among workers, participants with a higher *scn*-negative *S. aureus* carrier index (PR: 2.2, 95% CI: 1.04, 4.91) and a higher *S. aureus* CC9 nasal carrier index (PR: 1.3, 95% CI: 1.3, 5.3) were more likely to have reported symptoms of SSTI at the baseline visit. The relationships between nasal carrier indices and reports of SSTI at baseline trended upwards for MDRSA for all participants and for workers, specifically, but these associations were not significant at the p=0.05 level.

Associations between nasal carriage indices for *S. aureus*-related outcomes and biweekly reports of symptoms of SSTI are presented in **Table 4.5**. Symptoms of SSTI were assessed less comprehensively over the course of the four month study compared to the baseline visit. Most relationships between nasal carrier indices and ever-reported SSTI during the four month study trended downwards. Among workers, participants with a higher *scn*-negative *S. aureus* carrier index (PR: 0.3, 95% CI: 0.12, 0.87) and a higher tetracycline-resistant *S. aureus* nasal carrier index (PR: 0.5, 95% CI: 0.23, 0.93) were less likely to have reported symptoms of SSTI over the four month study.

Within-household transmission of *S. aureus spa* types over the four month period

We conducted 378 study visits with 53 households that contained both workers and household contacts (worker-only households are exempted from these counts; households where some participants completed less than 6/8 bi-weekly visits are included). We detected the same *S. aureus spa* type among workers and their household members during 11% of study visits (43/378 study visits). Characteristics of concordant *S. aureus spa* types and the frequency of their detection are described in **Appendix C: Table C2**.

Spa types t645 and t233, neither of which have previously been associated with livestock reservoirs, were the most frequently concordant *spa* types. We observed concordant *S. aureus spa* types with at least one indicator of livestock association (absence of *scn*, tetracycline resistance, CC398) between workers and their household contacts during nine study visits among nine households. In three households, we observed concordant *spa* types between workers and household members that did not share the same characteristics of livestock association. Concordant s*pa* types associated with *S. aureus* CC398 were observed twice among two households during the four month study, but a concordant *scn*-negative, tetracycline-resistant, CC398-associated *S. aureus spa* type was observed only once. We observed one instance of a concordant CC9-associated *S. aureus spa* type between workers and household members.

DISCUSSION

In this four month, repeated measures study of *S. aureus* nasal carriage among industrial hog operation workers and their household contacts, we observed persistent nasal carriage with MDRSA and *S. aureus* with indicators of livestock association (CC398, absence of *scn*, and tetracycline resistance) among both workers and their household contacts. Intermittent and persistent nasal carriage with *S. aureus* CC398 was observed as frequently among workers (12% and 4%, respectively) as among household members (8% and 5%, respectively), but persistent nasal carriage of *S. aureus* CC9 was only detected among workers (6%). Workers' nasal carriage outcomes and frequency of face mask use were predictors of their household contacts' nasal carriage outcomes over the course of the study. Our findings suggest that workers may be directly or indirectly sharing antibiotic-resistant *S. aureus*, including *S. aureus* with indicators of livestock association, with their household members. Overall, these results suggest that the temporal dynamics of livestock-adapted *S. aureus* nasal carriage among occupationally-exposed individuals and their household contacts in a region of the United States with a high density of intensive food animal production are different than previously observed.

In general, household members were more likely to be non-carriers and less likely to be intermittent or persistent carriers of *S. aureus* with indicators of livestock association for three of the four markers of livestock association that we considered in this study (absence of *scn*, resistance to tetracycline, and strain type CC9). These findings are in line with previous observational studies that have observed low transmissibility of *S. aureus* with indicators of livestock association from occupationally-exposed cohorts to their household members (Garcia-Graells et al., 2013; Smith et al., 2011; Verkade et al., 2014). However, the distribution of *scn*-negative, tetracycline-resistant *S. aureus* CC398 nasal carriage was not statistically different between workers and household members over the four month study: 86% and 92% were non-carriers, 10% and 4% were intermittent carriers, and 4% and 4% were persistent carriers, respectively. Although nasal carriage with CC398 was much less frequent among both workers and household members in this study compared to similar cohorts in Europe (Denis et al., 2009; Garcia-Graells et al., 2013), this study is the first to observe similar dynamics of persistent CC398 nasal carriage between individuals who are directly exposed to intensively-raised pigs and those who are only indirectly exposed.

There are several possible explanations for this finding. One explanation is that *S. aureus* CC398 is being spread from workers to household members through human-to-human transmission. However, if this were the case, we might have expected to see more instances of concordant *scn*-negative, tetracycline-resistant CC398-associated *S. aureus spa* types between workers and household members during bi-weekly study visits. Instead, we observed only one instance (t14157) – the same as the number of concordant CC9-associated *spa* types observed in this study, and CC9 was rarely observed among household members. However, we only examined one *S. aureus* nasal colonization isolate per person in this study. Thus, it is possible that some participants were colonized with multiple strains, and we missed some instances of *S. aureus* CC398 concordance. A second possible explanation for this finding is that workers are contaminating surfaces in their home environment with *S. aureus* CC398. *S. aureus* can survive and persist on environmental surfaces (Davis, Iverson, et al., 2012); humans can transfer these bacteria to their hands and then to the nose via touching. It is possible that *S. aureus* CC398 is better suited to survive on environmental surfaces than *S. aureus* with other indicators of livestock-association (e.g. CC9), although, to our knowledge, this has not been investigated. If environmental contamination is responsible for our results, the lag time between workers' transfer of *S. aureus* CC398 onto household environmental surfaces and household members' nasal contamination with these bacteria could explain why we infrequently observed concordant *scn*-negative, tetracycline-resistant CC398-associated *S. aureus spa* types between workers and household members during bi-weekly study visits. However, our study was not designed to investigate this hypothesis. A third explanation for this finding is environmental transmission of *S. aureus* CC398 into the home environment. These bacteria could enter the household environment in a region with a high density of intensive livestock production through bioaerosol emissions (Ferguson, 2012; Gibbs et al., 2006; Green et al., 2006), direct spraying of liquid manure (Casey, Curriero, et al., 2013), contaminated soil (Schulz et al., 2012), and contaminated insects or other pests (Ahmad et al., 2011; Van De Giessen et al., 2009). Household members could contaminate their noses with these bacteria through inhalation or through touching. Future studies of *S. aureus* nasal carriage dynamics in regions with high density of intensive livestock production should consider both occupational and environmental routes of exposure.

Among workers, within-person changes in the frequency of face mask use was a significant predictor of the bi-weekly presence of MDRSA and *S. aureus* with indicators of livestock association in a worker’s nose. Face masks can modify the dose of bioaerosols that enter a worker’s nose during exposure to the food animal production environment. In the United States. In this study, only 20% of workers reported always wearing a face mask throughout the four month study period (data not shown). Importantly, adults and minors who lived with workers who reported infrequent mask use had a higher percent of nasal swabs positive for *S. aureus* with indicators of livestock association over the four month period, relative to household members who lived with workers who reported always wearing face masks. This finding lends support to our hypothesis that livestock-adapted, antibiotic-resistant bacteria that enter workers’ noses through exposure to the livestock environment may be directly or indirectly shared with household members. Increased mask use could protect against this dissemination pathway.

Most predictors of the percent of workers’ nasal swabs positive for *S. aureus* and related outcomes over the four month study were related to household exposures, rather than occupational exposures. Having pets in the home, raising animals on the home property, and larger household size were all associated with more frequent nasal carriage of *S. aureus* with at least one indicator of livestock association. Interestingly, the more frequently household members were positive for *S. aureus* nasal carriage over the four month study, the more frequently a worker living in that household was positive for *S. aureus*. Simultaneously, the converse was true; the more frequently workers were positive for *S. aureus* nasal carriage over the four month study, the more frequently adults and minors living in their household were positive for *S. aureus*. This suggests that workers and their household contacts are exchanging *S. aureus*, either through person-to-person transmission or via shared fomites. We observed shared *S. aureus spa* types between workers and their household contacts on 43/378 separate study visits, which lends support to this finding. The transfer of *S. aureus* with indicators of livestock-association appeared to flow unidirectionally from workers to household members, however. The more frequently workers were positive for *scn*-negative *S. aureus* nasal carriage over the four month study, the more frequently adults and minors living in their household were positive for *scn*-negative *S. aureus*, but not the other way around. Shared *spa* types with matching indicators of livestock association were only observed on 6/378 visits.

Persistent nasal carriage of *S. aureus* with indicators of livestock association was lower among workers than has previously been observed among individuals with frequent contact with pigs. Thirteen percent of workers in this study persistently carried *scn*-negative *S. aureus*, 9% persistently carried tetracycline-resistant *S. aureus*, 4% persistently carried *S. aureus* CC398 (all were *scn*-negative and tetracycline-resistant), and 5% persistently carried *S. aureus* CC9 over the four month period. As we have observed in previous studies (Nadimpalli et al., 2014b; Rinsky et al., 2013), nasal colonization was dominated by MSSA, rather than MRSA. In contrast, Köck *et al*. reported 66% of 35 pig farmers persistently carried MRSA CC398 during a one month period including up to two weeks away from the hog farm environment (Köck, Loth, et al., 2012). Verkade *et al*. reported 23% of veterinarians, most of whom had frequent contact with pigs, persistently carried MRSA CC398 over a two-year period (Verkade et al., 2013). We recently conducted a pilot study in the same region as the present study in which we observed 46% of 22 industrial hog operation workers persistently carried *scn*-negative *S. aureus*, 36% persistently carried tetracycline-resistant *S. aureus*, 32% persistently carried *S. aureus* CC398, and 9% persistently carried *S. aureus* CC9 over a two-week period including up to 96 hours away from work (Nadimpalli et al., 2014b). The percent of persistent carriers of most of these outcomes was much lower in the present study; however, persistent *S. aureus* nasal carriage can appear more common when assessed over shorter periods of time (Vandenbergh et al., 1999). Indeed, we observed many more intermittent carriers of these outcomes in the present study than in the two-week study. Truly persistent nasal colonization with *S. aureus* with indicators of livestock association appears to be less common among this study population than other regions of the world (Garcia-Graells et al., 2013; Verkade et al., 2013). Overall, the distribution of persistent, intermittent, and non-carriers of *S. aureus* in this study population does not differ from healthy populations in the United States (Muthukrishnan et al., 2013).

Persistent nasal carriers of *S. aureus*-related outcomes in this study had several fold higher *S. aureus* $\log_{10}$CFU in their noses relative to the null than intermittent carriers. Using fixed effects models, in which each individual serves as his or her own control, we were not able to identify time-varying occupational or personal exposures that were associated with a higher quantity of *S. aureus* within workers' noses. It may be that we did not have sufficient power to identify time-variant exposures related to workers' *S. aureus* nasal loads, or that time-invariant characteristics that we did not or were unable to measure (e.g. innate biological or immune characteristics) are predictors of the higher bacterial load observed among persistent *S. aureus* nasal carriers (Peacock et al., 2001). This study was not designed to assess time-varying exposures that may have affected the quantity of *S. aureus* within household members' noses. Determinants of persistent versus intermittent *S. aureus* nasal carriage in the general population have not been established, but are thought to be related to inherent biological or immunological characteristics (Peacock et al., 2001; Van Belkum et al., 2009). Cross-sectional studies that seek to examine quantitative *S. aureus* as an outcome should consider that variation in *S. aureus* CFU between participants may largely be attributable to whether participants are intermittent or persistent carriers of *S. aureus*, which may be difficult to determine based on a single nasal swab. Indeed, using fixed effects models, we did not find evidence of positive relationships between exposure to wean pigs or giving pigs shots and amount of *S. aureus* in the nose, relationships that we observed in a cross-sectional analysis of this cohort (**Chapter 3**).

Among workers, we observed an incidence rate of symptoms of SSTI over the four month study that was similar to what we expected based on workers' reports of symptoms of SSTI during the baseline study visit (**Chapter 3**). At the baseline study visit, six workers reported symptoms of SSTI in the three months prior. Assuming workers developed these symptoms 1.5 months prior to baseline (on average), the incidence rate of symptoms of SSTI over the three months prior to baseline was 22.6 cases /1,000 person-months. Over the four month period, we observed an incidence rate of symptoms of SSTI of 20.2 cases/1,000 person-months, similar to baseline results. We observed some indication that workers who reported symptoms of SSTI at baseline were more frequent carriers of *S. aureus* with indicators of livestock association (absence of *scn*, *S. aureus* CC9) during the four month study, although workers who reported symptoms of SSTI during the course of the four months study were less frequent carriers of *S. aureus* with indicators of livestock association (absence of *scn*, tetracycline-resistant *S. aureus*). The rate of symptoms of SSTI in this population is much higher than the rate of SSTI previously reported among individuals with livestock exposure in the United States (6.6 cases/1,000 person-months) (Wardyn et al., 2015). However, the study by Wardyn *et al*. was among farm owners and operators, for whom livestock exposure is much lower than farm workers. A discussion regarding why the incidence of reported symptoms of *S. aureus* infection in this study population may not estimate the true prevalence of *S. aureus* infection in this population is provided elsewhere (**Chapter 3**).

We observed far fewer reports of symptoms of SSTI among household members during the four month study as predicted based on the prevalence of reports of recent SSTI at the baseline visit (**Chapter 3**). At baseline, 6/80 household members reported symptoms of SSTI during the three months prior. Assuming household members developed these symptoms 1.5

months prior to baseline (on average), we would have expected an incidence rate of symptoms of SSTI of around 24 cases/1,000 person-months during the four month study; instead, we observed a rate of 7.0 cases/1,000 person-months. There are two explanations for this result. First, it is possible that adults and minors in particular over-reported symptoms of recent SSTI during the baseline visit. Possible methodological and participant-centered reasons for over-reporting are discussed elsewhere (**Chapter 3**). Second, it is possible that household members underestimated symptoms of SSTI during bi-weekly study visits. In the bi-weekly questionnaire, we asked participants to report symptoms of SSTI as part of a larger section that assessed the severity of symptoms of general ill-health. Because symptoms of SSTI were not assessed separately, it is possible that participants (particularly minors) did not consider whether they were experiencing symptoms of SSTI as thoroughly as they did when completing the baseline questionnaire. Further, we asked participants to report symptoms of *current* SSTI during bi-weekly study visits, whereas we asked participants to report symptoms of current *or prior* symptoms of SSTI during the baseline visit. There may be greater stigma associated with reporting current infection compared to past infection (Perry & Donini-Lenhoff, 2010). Overall, differences in our survey tools and participants' potential concerns about reporting current versus prior infections may be related to the discrepancy in household members' reported symptoms of SSTI between baseline and bi-weekly visits.

Using fixed effects models where individuals serve as their own controls, we observed that high barn temperatures the week prior to nasal swabbing were associated with a reduction in the quantity of *S. aureus* we recovered from participants' noses. This is consistent with previous work that has observed a reduction in aerial bacterial and dust levels in confinement barns with increases in temperature (Curtis et al., 1975). High barn temperatures did not affect the presence or absence of *S. aureus* in a worker's nose. However, future work that evaluates quantitative measures of *S. aureus* nasal carriage among individuals exposed to confinement barns should consider the effect of seasonal temperature on the presence of *S. aureus* in the nose, as we did here.

There are several limitations to this study. First, we did not collect samples from intensively-raised food animals or confinement barns. These samples would have increased our certainty that *S. aureus* with indicators of livestock association that we recovered from participants' noses was originating from the industrial hog farm environment. Theoretically, since most workers who enrolled in this study identified as Hispanic (89%), it is possible that the *S. aureus* characteristics and strain types we detected among workers were unrelated to livestock exposure and were instead unique to this ethnic group; however, we observe no evidence in the literature to support this. In particular, animal or environmental source samples would have helped us confirm a livestock source for *S. aureus* CC9 in the United States, as livestock reservoirs for *S. aureus* CC9 have only previously been described in Asia. Further research is needed to confirm that *S. aureus* CC9 is a specific marker for livestock-adapted *S. aureus*. Second, the majority of workers' nasal swabs (678/790) were collected less than 24 hours since last work shift at an industrial hog operation. This length of time is not microbiologically relevant to examine associations between time away from work and reductions in the presence or amount of *S. aureus* in workers' noses, one of the main goals of our study. Third, as with previous studies we have conducted in this region of the United States (Nadimpalli et al., 2014b; Rinsky et al., 2013), industrial hog operation workers and household members were recruited via snowball sampling, rather than via random sampling from an enumerated population (e.g. employee records). Thus, it is unclear whether our findings are generalizable to all livestock workers in the United States. Finally, differences in survey instruments between the baseline and biweekly study visits made it difficult to estimate reliable rates of symptoms of SSTI among this population.

This study provides novel insights into the temporal dynamics of antibiotic-resistant *S. aureus* nasal carriage among industrial hog operations workers and their household contacts in a region with a high density of intensive livestock production. Interestingly, the dynamics of *S. aureus* CC9 nasal carriage among participants in this study closely resembled the dynamics of CC398 nasal carriage among occupationally-exposed cohorts and household contacts described in Europe (Graveland et al., 2011; Verkade et al., 2014). CC9 has previously been described among livestock and livestock workers in Asian countries (Fang et al., 2014), and only infrequently among livestock reservoirs elsewhere. Overall, these findings suggest that the global epidemiology of livestock-adapted *S. aureus* may be changing to become less region-specific, and that livestock-adapted *S. aureus* in some regions of the United States may be shared with individuals without direct livestock contact.

TABLES

Table 4.1. Predictors of persistence of nasal carriage with S. aureus and related outcomes among 98 hog operation workers sampled bi-weekly over four months in North Carolina, 2013-2014.[a]

	S. aureus carrier index (%)	MDRSA carrier index (%)	*scn*-negative *S. aureus* carrier index (%)	*S. aureus* CC398 carrier index (%)	*S. aureus* CC9 carrier index (%)
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
Number of pets at home (in increments of 2)	4.0 (-2.8, 10.8)	4.0 (0.3, 7.7)	1.8 (-1.0, 4.7)	4.9 (2.0, 7.8)	0.4 (-6.1, 6.9)
Animals raised on property (yes/no)	10.1 (-9.6, 29.8)	5.0 (-9.0, 18.9)	8.1 (-5.8, 22.0)	13.5 (0.1, 26.8)	4.6 (-10.4, 19.5)
Number of individuals living in household (in increments of 2)	8.7 (-1.7, 19.1)	1.3 (-7.2, 9.7)	7.5 (0.0, 14.9)	2.3 (-4.5, 9.2)	4.9 (0.2, 9.7)
Average nasal carrier index for outcome among household members living in household (in increments of 10)[b]	5.2 (3.5, 6.9)	-0.2 (-0.5, 0.1)	0.7 (-0.9, 2.2)	0.5 (-0.8, 1.8)	-1.9 (-4.4, 0.6)
Years worked at any industrial hog operation (in increments of 10)	11.7 (-2.9, 26.4)	10.5 (-0.9, 21.8)	11.4 (1.6, 21.1)	3.3 (-2.2, 8.7)	6.3 (-3.1, 15.7)
Frequency of face mask use at work					
Never	6.5 (-17.8, 30.8)	22.0 (1.9, 42.0)	19.6 (1.8, 37.3)	4.6 (-6.7, 15.8)	10.0 (-5.6, 25.6)
Sometimes	8.4 (-8.3, 25.0)	12.9 (-0.6, 26.5)	11.7 (-1.5, 24.9)	7.3 (-1.7, 16.3)	6.3 (-3.9, 16.4)
Always	ref	ref	ref	ref	ref

[a]Estimated with linear regression models using a generalized estimating equation with an exchangeable correlation matrix to account for the non-independence of observations within households.

[b]For example, we examined whether the average *S. aureus* nasal carrier index among household member(s) living in a household (in increments of 10) predicted the *S. aureus* nasal carrier index of a worker living in that household.

Table 4.2. Predictors of persistence of nasal carriage with *S. aureus* and related outcomes among 77 household contacts sampled bi-weekly over four months in North Carolina, 2013-2014.[a]

	S. aureus carrier index (%)	*scn*-negative *S. aureus* carrier index (%)	tetracycline-resistant *S. aureus* carrier index (%)
	β (95% CI)	β (95% CI)	β (95% CI)
Average nasal carrier index for outcome among workers living in household (in increments of 10)[b]	6.3 (4.0, 8.6)	3.0 (0.9, 5.2)	1.1 (-0.2, 2.4)
Lives with worker(s) who never wear face mask at work	23.9 (-1.9, 49.7)	18.8 (2.2, 35.4)	14.6 (1.3, 28.0)
Lives with worker(s) who sometimes or never wear face mask at work	2.7 (-19.2, 24.6)	9.9 (1.7, 17.8)	7.4 (0.6, 14.3)

[a]Estimated with linear regression models using a generalized estimating equation with an exchangeable correlation matrix to account for the non-independence of observations within households.

[b]For example, we examined whether the average *S. aureus* nasal carrier index among worker(s) living in a household (in increments of 10) predicted the *S. aureus* nasal carrier index of a household member living in that household.

Table 4.3. Associations between average within-worker changes in personal and occupational exposures the week prior to nasal sampling and average within-worker changes in nasal carriage of *S. aureus* and related outcomes, as measured among 98 industrial hog operation workers sampled bi-weekly over four months in North Carolina, 2013-2014.

	S. aureus		MDRSA		tetracycline-resistant *S. aureus*		*S. aureus* CC9	
	OR (95% CI)[a]	β (SE)[b]	OR (95% CI)[as]	β (SE)[b]	OR (95% CI)[a]	β (SE)[b]	OR (95% CI)[a]	β (SE)[b]
Hours worked (in increments of 10 hours)	1.1 (0.9, 1.5)	0.06 (0.06)	0.8 (0.4, 1.5)	0.00 (0.01)	1.2 (0.8, 1.8)	0.01 (0.04)	1.5 (1.0, 2.2)	0.01 (0.04)
Barn temperature								
Hot	1.4 (0.6, 3.2)	-0.41 (0.18)	0.5 (0.1, 1.5)	-0.21 (0.13)	0.7 (0.2, 2.0)	-0.13 (0.13)	0.7 (0.3, 1.7)	-0.17 (0.12)
Cold or Comfortable	ref		ref		ref		ref	
Frequency of face mask use at work								
Never	1.2 (0.4, 3.3)	0.01 (0.19)	3.5 (0.9, 13.9)	0.21 (0.14)	1.5 (0.4, 4.9)	-0.01 (0.13)	2.1 (0.8, 5.9)	0.19 (0.12)
Sometimes	1.6 (0.7, 3.5)	-0.11 (0.17)	4.7 (1.5, 15.4)	-0.04 (0.14)	4.7 (1.5, 14.1)	0.16 (0.11)	3.8 (1.4, 10.5)	0.09 (0.11)
Always	ref		ref		ref		ref	

[a]Estimated using conditional fixed effects logistic regression models to account for repeated measurements from the same individuals.

[b]Estimated using conditional fixed-effects linear regression models to account for repeated measurements from the same individuals.

Table 4.4. Associations between persistence of nasal carriage with *S. aureus* and related outcomes over four months and reported skin and soft tissue infections in the three months prior to baseline among 98 industrial hog operation workers and 77 household contacts in North Carolina, 2013-2014.[a,b,c]

	Reported symptoms of SSTI during the three months prior to the baseline visit		
	Overall (N=12)	Workers (N=6)	Minors (N=6)
	PR (95% CI)	PR (95% CI)	PR (95% CI)
S. aureus carrier index	1.9 (1.1, 3.2)	2.3 (0.8, 6.2)	1.3 (0.7, 2.4)
MDRSA carrier index	1.6 (0.9, 2.8)	2.0 (1.0, 4.0)	0.8 (0.2, 3.0)
scn-negative *S. aureus* carrier index	1.6 (0.9, 2.9)	2.2 (1.0, 4.9)	0.6 (0.2, 2.4)
tetracycline-resistant *S. aureus* carrier index	0.4 (0.1, 1.2)	0.6 (0.2, 1.5)	--
S. aureus CC398 carrier index	--	--	--
S. aureus CC9 carrier index	2.1 (1.1, 3.8)	2.6 (1.3, 5.3)	--

[a]Outcome was defined based on participant responses to the baseline questionnaire. Outcome was coded "Yes" for participants if they reported "Yes, in the past three months" to any of the following: *S. aureus* infection; skin boil; pus-filled abscess; red, painful, swollen skin bump or "pimple"; or spider bite that is itchy. Participants were shown pictures of *S. aureus* skin infections with each of these presentations prior to answering this question. This variable was coded "No" for participants if they reported "No" to all of the above categories. Otherwise, this question was coded as missing.
[b]Nasal carriage indices were examined in increments of 40%.
[c]Estimated with log binomial regression models using a generalized estimating equation with an exchangeable correlation matrix to account for the non-independence of observations within households.

Table 4.5. Associations between persistence of nasal carriage with *S. aureus* and related outcomes and ever-reported symptoms of skin and soft tissue over four months among 98 industrial hog operation workers and 77 household contacts in North Carolina, 2013-2014.[a,b,c]

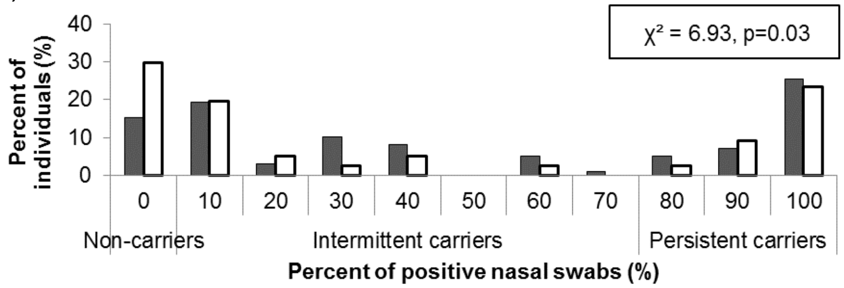
	Reported symptoms of SSTI during the four month study		
	Overall (N=9)	Workers (N=7)	HH Members (N=2)
	PR (95% CI)	PR (95% CI)	PR (95% CI)
S. aureus carrier index	0.6 (0.3, 1.1)	Does not converge	1.4 (0.4, 5.4)
MDRSA carrier index	0.6 (0.3, 1.1)	0.5 (0.2, 1.0)	0.7 (0.2, 2.2)
scn-negative *S. aureus* carrier index	0.4 (0.2, 1.1)	0.3 (0.1, 0.9)	Does not converge
tetracycline-resistant *S. aureus* carrier index	0.6 (0.4, 1.1)	0.5 (0.2, 0.9)	1.1 (0.4, 2.9)
S. aureus CC398 carrier index	--	--	Does not converge
S. aureus CC9 carrier index	1.0 (0.5, 1.8)	0.7 (0.4, 1.4)	Does not converge

[a]Outcome was defined based on participant responses to the baseline questionnaire. Outcome was coded "Yes" for participants if they reported "Yes, in the past three months" to any of the following: *S. aureus* infection; skin boil; pus-filled abscess; red, painful, swollen skin bump or "pimple"; or spider bite that is itchy. Participants were shown pictures of *S. aureus* skin infections with each of these presentations prior to answering this question. This variable was coded "No" for participants if they reported "No" to all of the above categories. Otherwise, this question was coded as missing.
[b]Naal carriage indices were examined in increments of 40%.
[c]Estimated with log binomial regression models using a generalized estimating equation with an exchangeable correlation matrix to account for the non-independence of observations within households.

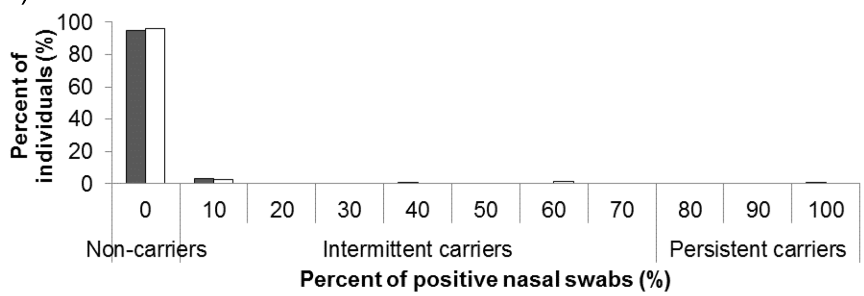
FIGURES

Figure 4.1. Distribution of *S. aureus*, MRSA, and MDRSA nasal carriage indices among 98 industrial hog operation workers and 77 household contacts cultured bi-weekly over four months in North Carolina, 2013-2014.[a,b,c]

a) *S. aureus*



b) MRSA



c) MDRSA

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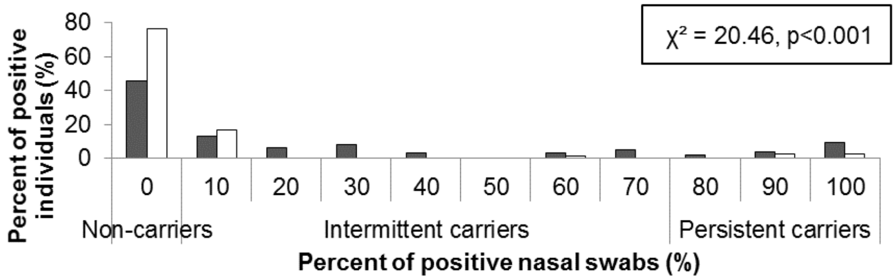
[a]Carrier index is defined as the percent of nasal swabs positive for an outcome for each individual. Individuals with no swabs positive for an outcome were defined as non-carriers of that outcome. Individuals with >0% and <80% of swabs positive for an outcome were intermittent carriers. Individuals with ≥80% of swabs positive for an outcome were persistent carriers.

[b]Results from participants who completed at least 6/8 bi-weekly follow-up study visits are presented.

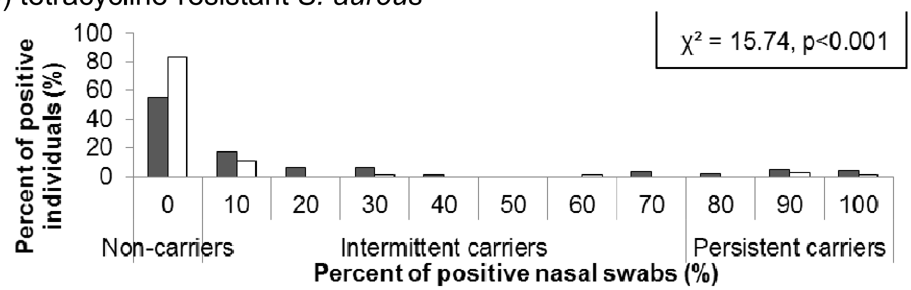
[c]For each outcome, the Rao-Scott chi-square test was used to examine the hypothesis that the distribution of non-carriers, intermittent carriers, and persistent carriers did not differ between industrial hog operation workers and household contacts, while accounting for within-household clustering. Some tests could not be computed because at least one cell had a frequency of 0. Degrees of freedom=2 for all.

Figure 4.2. Distribution of nasal carriage indices of *S. aureus* with indicators of livestock association among 98 industrial hog operation workers and 77 household contacts cultured bi-weekly over four months in North Carolina, 2013-2014.[a,b,c]

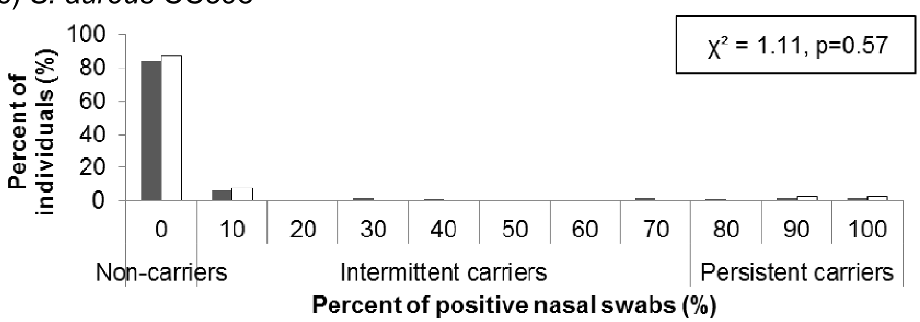
a) *scn*-negative *S. aureus*



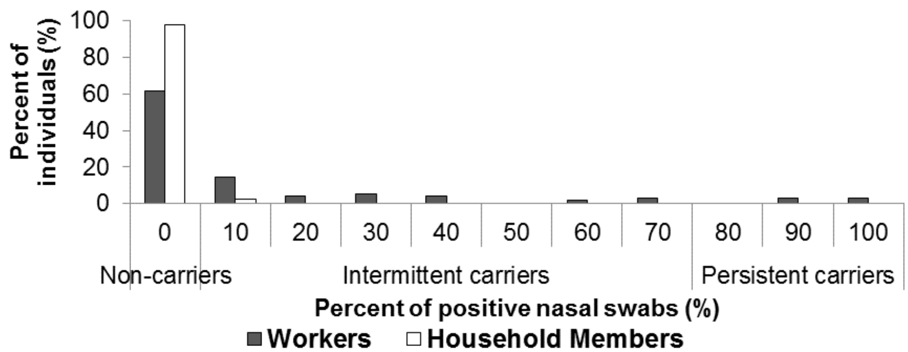
b) tetracycline-resistant *S. aureus*



c) *S. aureus* CC398[d]



c) *S. aureus* CC9



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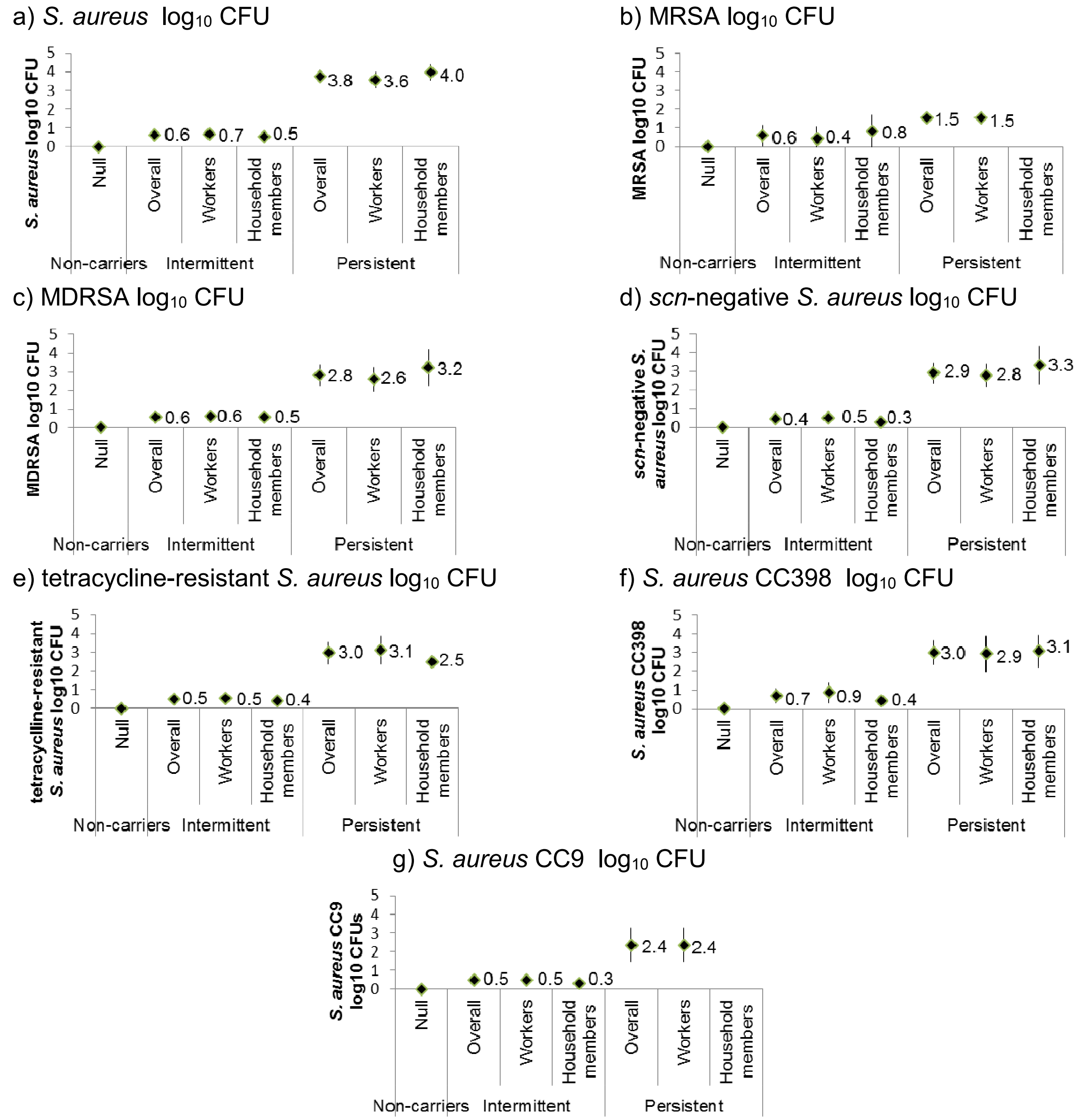
[a]Carrier index was defined as the percent of nasal swabs positive for an outcome for each individual. Individuals with no swabs positive for an outcome were defined as non-carriers of that outcome. Individuals with >0% and <80% of swabs positive for an outcome were intermittent carriers. Individuals with ≥80% of swabs positive for an outcome were persistent carriers.

[b]The Rao-Scott chi-square test was used to examine the hypothesis that the distribution of non-carriers, intermittent carriers, and persistent carriers did not differ between industrial hog operation workers and

household contacts for each outcome, while accounting for within-household clustering. Some tests could not be computed because at least one cell had a frequency of 0. Degrees of freedom=2 for all.
[c]Distributions of persistent, intermittent and non-carriers of CC398 were similar between workers and household members even when comparing CC398 with one or more indicators of livestock association. Rao-Scott chi-square test results are provided in Appendix C: Table C1.

Figure 4.3. Average difference in *S. aureus* colony forming units (CFU) among persistent and intermittent nasal carriers of *S. aureus* and related outcomes relative to non-carriers, 2013-2014.[a,b,]



[a]Average *S. aureus* $\log_{10}$ CFU were estimated relative to non-carriers with linear regression models using a generalized estimating equation with an exchangeable correlation matrix to account for the non-independence of observations within individuals and within households. Methods for imputing $\log_{10}$CFU are provided in the Statistical Analysis section.

[b]Overall analytical sample size=1,459 nasal swabs.

CHAPTER FIVE: RECOVERY OF ANTIBIOTIC-RESISTANT *STAPHYLOCOCCUS AUREUS* FROM INDUSTRIAL HOG OPERATION WORKERS' HOUSEHOLDS

SUMMARY

Objective: Antibiotic-resistant *S. aureus*, including methicillin-resistant *S. aureus* (MRSA) and multidrug-resistant *S. aureus* (MDRSA) has emerged among intensively-raised hogs. We aimed to evaluate whether industrial hog operation (IHO) workers' households were contaminated with antibiotic-resistant *S. aureus*, and whether environmental exposure to IHOs was associated with household contamination. Methods: This study was nested within a four-month study of *S. aureus* nasal carriage among IHO workers and household contacts. We assessed surface samples from volunteer households for *S. aureus*, including *mec*A (indicating MRSA), resistance to ≥3 antibiotic classes (indicating MDRSA), absence of *scn* (indicating livestock association), and strain type. We quantified households' exposure to IHOs based on proximity and the number and type of animals grown on IHOs. We used linear regression to examine associations between households' exposure to IHOs and the percentage of household surfaces contaminated with *S. aureus* and related outcomes. Results: Twenty-six households participated. *S. aureus* was detected in 23/26 households, MDRSA in 11/26, and MRSA in 3/26. Higher environmental exposure to IHOs was associated with a larger percent of household surfaces positive for MDRSA ($p<0.05$) and *S. aureus* CC398 ($p<0.05$). Discussion: This study provides evidence that livestock-associated strains of *S. aureus* can be found in the households of IHO workers, and suggests that environmental transport may play a role in their contamination.

INTRODUCTION

Antibiotic-resistant *Staphylococcus aureus* has been observed among livestock, poultry, and other domesticated animals across the globe. These antibiotic-resistant *S. aureus* strains, which include methicillin-resistant (MRSA) and multidrug-resistant *S. aureus* (MDRSA), are commonly referred to as "livestock-associated" and can be transmitted from colonized animals to individuals in close contact with these animals. Nasal carriage of these bacteria among humans does not necessarily lead to infection, although skin and soft tissue infections among healthy livestock workers has been observed (Wardyn et al., 2015). In European countries, most livestock-associated *S. aureus* belong to clonal complex (CC) 398, while in Asian countries, livestock-associated *S. aureus* primarily belong to CC9.

There are several pathways by which antibiotic-resistant *S. aureus* could disseminate from the industrial hog operation environment to people and their communities (**Figure 5.1**). Transmission to occupationally-exposed individuals (e.g. livestock workers, veterinarians) is well-documented (Garcia-Graells et al., 2012; Smith et al., 2011), and there is some evidence that occupationally-exposed individuals can transfer these bacteria to their household members (Bosch et al., 2013). However, contamination of the household environment could also play a role in the transmission of livestock-associated *S. aureus*. In regions with a high density of industrial hog operations in particular, the household environment could become contaminated with livestock-associated *S. aureus* via airborne particles (Gibbs et al., 2006), soil tracked inside (Schulz et al., 2012), or insects carrying these bacteria (Ahmad et al., 2011). Contamination could also occur through contact with colonized individuals' noses, skin, or clothing, including clothing worn in a confinement barn. The household environment could thus serve as a reservoir for colonization or infection with these bacteria, even when occupationally-exposed individuals are away from the industrial hog operation environment. Previous research has implicated the household environment as a reservoir for colonization and infection with hospital and community-associated *S. aureus* (Kniehl et al., 2005; Uhlemann et al., 2011), and antibiotic-resistant, livestock-associated *S. aureus* has previously been recovered from the household environment (Garcia-Graells et al., 2013). However, the potential for the household environment to serve as a reservoir for livestock-associated *S. aureus* in a region with a high density of intensive food animal production has never before been examined.

We conducted a pilot household environmental sampling study to investigate whether the household environment could serve as a reservoir for livestock-associated *S. aureus*. Specifically, we examined: (a) the prevalence of household environmental contamination with *S. aureus* and related outcomes in a region with a high density of industrial hog operations; (b) associations between relative geographic exposure to these operations and the percent of household environmental surfaces contaminated with *S. aureus* and related outcomes; and (c) concordance between *S. aureus spa* types recovered from the household environment and *S. aureus spa* types recovered from the noses of household members ≤ two weeks prior to household environmental sampling.

MATERIALS AND METHODS

Data collection occurred between January, 2014 and June, 2014 by researchers from the University of North Carolina at Chapel Hill (UNC) with community organizers from the Rural Empowerment Association for Community Help (REACH).

Study design

This study was nested within a four-month cohort study of *S. aureus* nasal carriage among industrial hog operation workers and household members in eastern North Carolina, described in **Chapters 3 and 4**. Any household that was enrolled in the four-month cohort study was eligible to participate in the household environmental sampling study, provided all household members had missed no more than one bi-weekly follow-up nasal swab and questionnaire at the time of enrollment for the household environmental sampling study.

The first round of enrollment for the household environmental study began three months into the four-month study. At the time of consideration for inclusion, households were characterized as (a) “persistently-carrying” households, for which ≥80% of nasal swabs contributed by participants over the bi-weekly follow-ups thus completed were positive for *S. aureus;* (b) “intermittently-carrying” households, for which some but less than 80% of nasal swabs contributed by participants over the follow-ups thus completed were positive for *S. aureus;* and (c) “never-carrying” households, for which all nasal swabs contributed by participants over the follow-ups thus completed were negative for *S. aureus*. We invited a distribution of persistently-carrying and intermittently-carrying households to participate in the surface sampling study that approximated the distribution of such households in the four-month study; more never-carrying households were asked to participate in the environmental sampling study than their relative frequency in the four-month study. Thirty households were invited to participate on a rolling basis within one month before or after the end of the four-month cohort study.

Data collection

Surface wipe samples were collected from household environmental surfaces using autoclaved dry electrostatic cloths (Swiffer; Proctor & Gamble), based on methods from Davis *et al* (Davis, Baron, et al., 2012). Wipe samples were standardized to one ft^2 surface areas where possible. In each house, three types of sample sites were targeted: common touch sites, such as television remotes, refrigerator handles, microwave handles, and kitchen faucets; sites with heavy face and nose contact, such as bed pillows and living room couch pillows; and at least one air deposition site, such as the top of a bookshelf, top of a refrigerator, or the back of a television stand. Samples from air deposition sites were meant to capture *S. aureus* that had passively settled out of the air column in the household environment. One blank wipe sample was collected per household by momentarily exposing an autoclaved cloth to the ambient air. Individual cloths were stored in sterile WhirlPak plastic bags at room temperature away from light until analysis in the laboratory, up to three days later.

The number of individuals living in each household, the presence of pets inside the household, and whether or not pets were allowed on household furniture (living room sofas or beds) were noted at the time of household sampling.

Identification of *S. aureus* and MRSA

Upon arrival in the laboratory, 60 mL Mueller-Hinton broth containing 6.5% NaCl was added to each sample bag (Davis, Baron, et al., 2012), massaged by hand, then incubated overnight at 37°C. To isolate presumptive *S. aureus*, a loopful of Mueller-Hinton broth was streaked onto Baird-Parker with Egg Yolk Tellurite Enrichment and incubated at 37°C for 24-48 hours. To isolate presumptive MRSA, one mL of Mueller-Hinton broth was transferred to nine mLs of tryptic soy broth containing 2.5% NaCl, 75 mg/L aztreonam, and 3.5 mg/L cefoxotin (Krause, 2009) and incubated overnight at 37°C. This selective enrichment step is critical in isolating MRSA from environmental surfaces, as the bacteria may be few in number. Following incubation, a loopful of tryptic soy broth was streaked onto Baird-Parker and incubated at 37°C for 24-48 hours. Colonies with morphology characteristic of *S. aureus* and MRSA were streaked to isolation on trypticase soy agar with 5% sheep blood (Remel Laboratories, Lenexa, KS) and confirmed through catalase testing and tube coagulase testing with rabbit plasma (BD BBL™, Franklin Lakes, NJ).

A crude DNA extraction was performed on each isolate using a protocol adapted from Reischl *et al*. (Reischl et al., 2000). Multiplex PCR was used to amplify the *spa*, *scn*, *mec*A, and *mec*C genes. LGA251 (provided courtesy of Dr. Meghan Davis, JHU) was used as an extraction and PCR control for *spa* and *mec*C, while a clinical MRSA isolate (courtesy of Dr. Jill Stewart, UNC) was used as an extraction and PCR control for *spa*, *scn*, and *mec*A. Sterile water was used a negative control. PCR products were visualized on 2% agarose gels stained with ethydium bromide. Colonies positive for *spa* were *S. aureus*; isolates positive for *spa* and either *mec*A or *mec*C were MRSA.

Antibiotic Susceptibility

One isolate per *S. aureus*-positive sampling site was assessed for susceptibility to a panel of 11 antibiotic classes, comprising 15 antibiotics, using the Kirby-Bauer disk diffusion method. Diameter interpretations were based on Clinical and Laboratory Standards Institute (CLSI) guidelines [27]. Inducible clindamycin resistance was evaluated in erythromycin-resistant isolates using the D-zone test [28]. Isolates that demonstrated complete resistance to three or more classes of antibiotics were classified as MDRSA [30].

Molecular analyses

The *spa* gene was amplified and sequenced for one isolate from each *S. aureus*-positive sampling site using methods described previously (European Union Reference Laboratory for Antimicrobial Resistance, 2009). All isolates were characterized by *spa* typing using the Ridom StaphType software and the Ridom SpaServer (http://spa.ridom.de/index.shtml). *spa* types were then assigned to putative MLST clonal complexes (CCs) 398, CC9, or neither CC based on the existing literature.

Indicators of livestock association

There are currently no established markers for livestock-associated *S. aureus*. We examined four indicators of livestock-association among *S. aureus* isolates: strain type CC398, strain type CC9, absence of *scn*, and tetracycline resistance. We have used CC398, absence of *scn*, and tetracycline resistance as indicators of livestock-association in previous work where rationale for these indicators is provided (Rinsky et al., 2013). We examined CC9 as a possible indicator of livestock association in this study due to increased reports among livestock herds and people in contact with these herds in several Asian countries (Asai et al., 2012; Fang et al., 2014; Patchanee et al., 2014; Wagenaar et al., 2009), and frequent observation of this strain type among industrial livestock operation workers from the same geographic region in which we conducted the present study (Rinsky et al., 2013). However, the specificity of CC9 as a marker of livestock association has not yet been demonstrated in the literature.

Determination of household-level relative exposure to industrial hog operations

Latitude and longitude coordinates for all hog operations issued general permits by the North Carolina Department of Natural Resources (NC DENR) as of 2003 were obtained from the NC DENR Division of Water Quality (http://data.nconemap.com/geoportal/) and imported into ArcGIS 10.1 (ESRI, Redlands, CA, USA). Hog operations for which general permits did not exist as of January 5, 2015 were excluded (http://portal.ncdenr.org/web/wq/animal-facility-map). NC DENR only issues permits for animal feeding operations; thus, pasture-raised hog farms were not included.

Twenty-two of 26 participating households provided their household addresses as part of baseline data collection for the four-month cohort study. Household addresses were queried in Google Maps to determine corresponding latitude and longitude coordinates, which were imported into ArcGIS 10.1. The "Point Distance" tool in ArcGIS was used to calculate the distances between each household and every industrial hog operation within 22,860 m (roughly the width of Duplin County in eastern North Carolina) of that household. Distance matrices were imported into SAS 9.3 along with information about each industrial hog operation listed in a household's distance matrix, including the number of animals raised on an operation, the operation's permit number, and the type of animal production system permitted (e.g. farrow-to-wean, wean-to-feeder). We used information about the number of animals on an operation, the animal production system, and estimates of the accumulated manure (gallons/animal/year) for types of swine production systems in North Carolina (Crouse et al., 2014), to calculate the total manure produced per year by permit. Within each household's distance matrix, we merged multiple permits issued for the same facility to obtain a total volume of manure produced per industrial hog operation per year. Manure production was used as a proxy for exposure to industrial hog operations; manure production is a better proxy than animal head count, since facilities with similar head counts can house pigs that vary considerably in age and size.

We calculated each household's relative environmental exposure to industrial hog operations using the following formula, modified from Pavilonis *et al*. (Pavilonis et al., 2013):

$E_{relative} = \sum \frac{V}{d^2}$

Where $E_{relative}$ is a household's relative environmental exposure to industrial hog operations, V is the volume of manure produced by an industrial hog operation (gallons/year), and d is the distance between a household and an industrial hog operation (meters).

We also calculated log-transformed each household's relative environmental exposure to industrial hog operations using the following formula, for the purposes of calculating prevalence ratios for binary outcomes.

$E_{relative} = \log \sum \frac{V}{d^2}$

As described by Pavilonis *et al*., the purpose of this metric is not to predict the actual concentrations of *S. aureus* emitted by industrial hog operations. Rather, this metric is intended to qualify households' relative exposure given the proximity and density of industrial hog operations in the region. Because distance to an industrial hog operation was related to household's exposure as an inverse square, proximal hog operations contributed exponentially more to a household's relative exposure than an operation further away. Thus, we did not constrain the radius within which we examined industrial hog operations contributions (22, 860 m) to distances at which airborne *S. aureus* has previously been reported downwind of industrial hog operations (100 -200 meters) (Ferguson, 2012; Gibbs et al., 2006; Green et al., 2006). To our knowledge, no previous study has measured airborne *S. aureus* downwind of an industrial hog operation to the point of extinction.

Statistical analysis

We examined the distribution of household-level characteristics within the study population, focusing on those that could be associated with household environmental contamination with *S. aureus* (e.g. number of workers living in the household, whether or not pets were allowed inside, whether or not a household member had recently been exposed to the hospital or another medical facility).

We calculated the proportion of households positive for *S. aureus* and related outcomes at at least one sampling site with the total number of households sampled as the denominator. We also calculated the proportion of households positive for *S. aureus* and related outcomes at an air deposition site (e.g. top of a bookshelf, top of a refrigerator, back of a television stand) with the total number of households sampled as the denominator. Within each household, we calculated the percentage of sampling sites positive for *S. aureus* and related outcomes, using the number of samples obtained from that household as the denominator (air deposition site sample included).

We examined whether a household's relative environmental exposure to industrial hog operations was predictive of the percentage of household environmental samples positive for *S. aureus* and related outcomes using univariate linear regression models. We also examined whether a household's relative environmental exposure to industrial hog operations was predictive of the presence of *S. aureus* and related outcomes at an air deposition site within that household using univariate log binomial regression models, where sample size allowed.

We examined similarities between *S. aureus spa* types recovered from the household environment and *S. aureus spa* types recovered from household members' noses less than or equal to two weeks prior to household sampling. *S. aureus* has been observed to survive on surfaces in a dormant state for weeks to months (Freeman-Cook et al., 2009; Watson et al., 1998). Since the survival time for *S. aureus* with characteristics of livestock association has not been comprehensively investigated, we chose a conservative survival window of two weeks. The frequency of each *S. aureus spa* type detected in the household environmental sampling study was tabulated. For those households for which bi-weekly nasal swabs for the four-month study had been collected less than two weeks prior to household environmental sampling, the frequency of each *S. aureus spa* type recovered from household members' noses was also tabulated. Instances of household-level concordance, where the same *S. aureus spa* type was recovered from a household's environment as was recovered from household members' noses are presented.

For those households for which bi-weekly nasal swabs for the four-month study had been collected less than two weeks prior to household environmental sampling, we used household members' nasal swab results to calculate household-level "carrier indices" for *S. aureus* and related outcomes. Each household-level carrier index was calculated by determining the percentage of participants that were positive for that outcome out of the total number of participants who contributed nasal swabs during the most recent visit (e.g. a household-level *S. aureus* carrier index of 0 indicated that all participants who contributed nasal swabs were negative for *S. aureus* nasal carriage during the most recent visit; a household-level *S. aureus* carrier index of 100 indicated that all participants who contributed nasal swabs were positive for *S. aureus* nasal carriage during the most recent visit). We also calculated worker-level carrier indices for each household by calculating the percentage of workers that were positive for *S. aureus* and related outcomes during the most recent visit. We used univariate linear regression models to examine the associations between household-level nasal carrier indices, worker-level carrier indices, and percentage of household environmental samples positive for *S. aureus* and related outcomes. We did not observe any household-level risk factors (including relative environmental exposure to industrial hog operations) that were associated with both the exposure and the outcome in these models. Thus, unadjusted effect measures are presented for these associations.

We explored whether other household level-risk factors that have previously been associated with presence of antibiotic-resistant *S. aureus* in the household environment (e.g. household size, number of livestock workers living in the household, cumulative hours per week worked by livestock workers in living in household, pets in the household,) were predictive of the percentage of household environmental samples positive for *S. aureus* and related outcomes. We used univariate linear regression models to examine these associations.

All statistical analyses were performed using SAS 9.3 (SAS Statistical Institute, Cary, NC).

RESULTS

Prevalence of *S. aureus*, MRSA, MDRSA, and related outcomes in households

Twenty-six of 77 eligible households from the four-month cohort study participated in the household environmental sampling study. Household characteristics are provided in **Table 5.1**. On average, eight environmental samples were collected per household (min: four samples; max: nine samples). Samples from air deposition sites were collected from 23/26 households.

The prevalence of households positive for *S. aureus* and related outcomes is described in **Table 5.2**. *S. aureus* was detected in almost all households (23/26), although MDRSA (11/26) and especially MRSA (3/26) were less common. *S. aureus* with one or more indicators of livestock-association was recovered from over half of households sampled (14/26). Tetracycline-resistant *S. aureus* was recovered most frequently (14/26), but *scn*-negative *S. aureus* (12/26), *S. aureus* CC398 (6/26) and *S. aureus* CC9 (4/26) were also recovered from households.

S. aureus was recovered from an airborne deposition site in over a third of households from which airborne deposition site samples were collected (9/23). MRSA, MDRSA, and *S. aureus* with one or more indicators of livestock-association (except CC9) were also detected at air deposition sites. However, *S. aureus* recovered from most airborne deposition sites lacked indicators of livestock-association (6/9) (**Table 5.2**).

The frequency of recovery of *S. aureus* and related outcomes from different environmental sample types is described in **Table 5.2**. Samples with heavy face or nose contact (e.g. personal pillows, living room sofas, and living room sofa pillows) were the most common type of environmental sample positive for *S. aureus* and most related outcomes. Only *S. aureus*

CC9 was recovered more commonly from samples that were frequently touched (e.g. refrigerator handles, TV remotes, microwave handles, and kitchen faucets) than samples with heavy face or nose contact. MRSA was not detected among any samples that were frequently touched.

Detailed molecular and phenotypic descriptions of *S. aureus* isolates recovered during this study are presented in **Appendix D: Table D1**.

Associations between households' environmental exposure to industrial hog operations and presence of *S. aureus* and related outcomes in the household environment

Among the 22 households that provided their household addresses, the average distance between a household and the closest industrial hog operation was 1,496 m (min: 432 m, max: 4,076 m). On average, five industrial hog operations were located within 2500 m of participants' homes (median: 6 operations, min: 1, max: 12); 16 industrial hog operations on average were located within 5000 m of participants' homes (median: 14 operations, min: 4, max: 35). The interquartile range (IQR) of households' relative environmental exposure was 22.9 units.

Associations between households' relative exposure to industrial hog operations and the percentage of household surfaces that were positive for *S. aureus* and related outcomes are presented in **Figure 5.2**.

An increase by the IQR of household' environmental exposure to industrial hog operations was associated with an 11% increase in the percent of household environmental samples positive for MDRSA ($p<0.05$). MDRSA contaminated 21/201 household surfaces sampled in this study; most MDRSA (12/21) had one of more characteristics of livestock association. An increase by the IQR of household' exposure to industrial hog operations was also associated with a 10% increase in the percent of household environmental samples positive for *S. aureus* CC398 ($p<0.05$) and a 10% increase in the percent of household environmental samples positive for tetracycline-resistant *S. aureus* (p=0.06), although the 95% confidence interval for this effect measure contained the null.

We did not observe any association between households' relative environmental exposure to industrial hog operations and the presence of *S. aureus* at airborne deposition sites in the household (data not shown). We did not have sufficient sample size to examine this relationship with other *S. aureus*-related outcomes, as there were too few airborne deposition sites from which these outcomes were recovered.

Concordance between *S. aureus spa* types from the household environment and participants' noses

We observed 16 unique *spa* types among *S. aureus* isolates recovered from household environmental samples collected in this study (**Figure 5.3**). For 12/26 households sampled, the same spa type(s) was detected in the household environment as was detected in household members' noses ≤2 weeks prior to household environmental sampling. *S. aureus spa* type t034 (associated with CC398) was the most common *spa* type to be concordant between household members' noses and the household environment. Nasal swab data for ≤2 weeks prior to household environmental sampling was not available for 7/26 households.

Associations between the recovery of *S. aureus*-related outcomes from the household environment and presence of these outcomes in household members' noses ≤2 weeks prior to household environmental sampling are presented in **Figure 5.4**. Presence of *S. aureus* in household members' noses was strongly associated with the percentage of household environmental surfaces contaminated with *S. aureus*. We also observed strong associations between presence of MDRSA in household members' noses and recovery of MDRSA from the household environment, presence of CC398 in household members' noses and recovery of CC398 from the household environment, and presence of tetracycline-resistant *S. aureus* in household members' noses and recovery of tetracycline-resistant *S. aureus* from the household environment. We observed a positive association between presence of *scn*-negative *S. aureus* in household members' noses and recovery of *scn*-negative *S. aureus* from the household environment; however, the 95% CIs for this effect measure contained the null.

We did not observe any associations between the recovery of *S. aureus*-related outcomes from the household environment and presence of these outcomes in workers' noses (specifically) ≤2 weeks prior to household environmental sampling (data not shown).

Other risk factors for presence of *S. aureus* and related outcomes in the household environment

We examined additional household-level risk factors that previous studies have associated with presence of *S. aureus* and related outcomes in the household environment (Bramble et al., 2011; Kniehl et al., 2005; Scott et al., 2008; Uhlemann et al., 2011). Associations between these risk factors and the percentage of household surfaces that were positive for *S. aureus* and related outcomes are presented in **Appendix D: Table D2.** Whether or not pets were allowed on furniture was the only fixed household-level risk factor we observed to be associated with the presence of *S. aureus*-related outcomes in the household environment. Occupational risk factors, such as the number of livestock workers living in a household and the cumulative number of hours worked by livestock workers living in a household, were not related to the percentage of household surfaces that were positive for *S. aureus* and related outcomes.

DISCUSSION

In this pilot household sampling study in a region with a high density of industrial hog operations, we observed that households with greater environmental exposure to industrial hog operations had a higher percentage of surfaces in their home contaminated with multidrug-resistant *S. aureus* and *S. aureus* CC398. We recovered *S. aureus*, MRSA, and MDRSA with one or more indicators of livestock-association from airborne deposition sites among 3/26 households in this study. Among half of the households sampled (13/26), we observed concordance between *S. aureus spa* types recovered from the household environment and *S.*

aureus spa types detected in the noses of household members up to two weeks prior to household sampling, although directionality of this exchange cannot be determined given the study design. To the best of our knowledge, this is the first study to report links between the presence of antibiotic-resistant *S. aureus* with indicators of livestock association in the household environment and nasal carriage of these bacteria among individuals living in the home.

Our findings suggest that communities proximal to industrial hog operations may be exposed to antibiotic-resistant *S. aureus* with indicators of livestock association, regardless of direct contact with livestock, through environmental exposure pathways. Previous studies have observed that individuals living in regions with a high density of industrial livestock operations are at a higher risk for nasal carriage and infection with antibiotic-resistant *S. aureus*, even when no contact with livestock is reported (Casey, Curriero, et al., 2013; Feingold et al., 2012; Lekkerkerk et al., 2015; Van Rijen et al., 2014). Some have speculated that human-to-human transmission from occupationally-exposed individuals to the larger community may be responsible for these findings (Lekkerkerk et al., 2012; Lekkerkerk et al., 2015; Van Rijen et al., 2014), although this hypothesis has limited support (Wassenberg et al., 2011). Others have speculated that individuals living near industrial livestock operations may be contaminated with livestock-associated *S. aureus* through environmental pathways, including air and waste emissions from these operations (Casey, Curriero, et al., 2013; Feingold et al., 2012). Our findings support this hypothesis. We observed a higher percentage of household surfaces contaminated with MDRSA and *S. aureus* with indicators of livestock association among households with greater potential relative environmental exposure to industrial hog operations. Although we could not conclude whether *S. aureus* in the household environment contributed to *S. aureus* nasal carriage among household members (despite observing *spa* type concordance in 13/26 households), other studies have observed that occurrence of *S. aureus* in the household environment is associated with intra-household *S. aureus* transmission (Knox et al., 2012). Together, our findings indicate that dissemination of livestock-associated *S. aureus* into surrounding communities via environmental pathways may contribute to nasal colonization in these populations.

The presence of *S. aureus* CC398 in the household environment may provide new insight into the “contamination versus colonization” hypothesis for human nasal carriage with livestock-associated *S. aureus*. Several studies have examined persistence of nasal carriage with *S. aureus* CC398 (and *S. aureus* with other indicators of livestock-association) among individuals with frequent, direct contact with livestock (e.g. livestock workers, veterinarians). Many of these studies have interpreted persistent nasal carriage with livestock-associated *S. aureus* during extended time away from work to mean that livestock-associated *S. aureus* can colonize the human nose, rather than simply contaminate it at work via inhaled dust and airborne particles (Köck, Loth, et al., 2012; Verkade et al., 2013). However, our findings indicate that the household environment could serve as an additional reservoir for livestock-associated *S. aureus* nasal carriage. Thus, even when away from the industrial hog production environment, workers could repeatedly re-contaminate their noses with livestock-associated *S. aureus* picked up from household surfaces, which may not be distinguishable from true colonization when *S. aureus* nasal carriage is assessed as a binary outcome (present vs. absent) over time. We did not investigate this hypothesis directly in the present study. Still, given these results, experimental studies rather than observational cohort studies among individuals with frequent livestock exposure may provide stronger insight into the “colonization versus contamination” hypothesis for *S. aureus* with indicators of livestock-association.

Households sampled in this study were located beyond a radius for which *S. aureus* dissemination from industrial hog operations has previously been examined. Hogs grown on industrial farm operations are colonized with antibiotic-resistant bacteria that can be emitted into the surrounding environment (Schulz et al., 2012) through ventilation fans, via pests, or as a result of waste disposal practices (in North Carolina, spraying waste on proximal fields). Studies in the United States have observed airborne *S. aureus* plumes downwind of industrial hog operations up to 215 meters (Ferguson, 2012; Gibbs et al., 2006; Green et al., 2006), but they did not measure further. *S. aureus spa* types isolated from the industrial hog farm environment have also been recovered from soil samples 300 m downwind of these operations (Schulz et al., 2012). On average, households in this study were approximately 1,500 m away from the closest industrial hog operation (min: 432 m, max: 4,076 m). Although these distances are greater than what has previously been explored, previous work examining *S. aureus* emissions from industrial hog farm operations have not tracked emissions across increasing distances to the point of non-detection. Thus, these bacteria may travel much further than has previously been described. The model we used in this study to examine households' exposures to antibiotic-resistant *S. aureus* does not provide insight into the distance *S. aureus* may travel from point sources. Despite a small sample size, we observed associations between households' environmental exposure to industrial hog operations percentage of household surfaces contaminated with MDRSA and *S. aureus* CC398 at these representative distances.

We used a qualitative metric that accounted for both proximity to and density of hog operations in our assessment of households' relative environmental exposure to industrial hog operations. However, our assessment of households' environmental exposure was relatively simplistic. We did not account for wind speed or direction, ambient temperature or humidity, season (related to UV light), or temporal information about the source (e.g. when ventilation fans were on or off, when liquid manure spraying was occurring). These factors would have improved our spatial and temporal understanding of households' environmental exposure to industrial hog operations, but this information was not collected or was unavailable for this region. Further, we did not account for households' proximity to and density of other food animal production operations located in the region. Turkey production is concentrated in eastern North Carolina (Webb, 2015), as are other types of intensive animal farming. However, spatial information describing other types of confined food animal operations in North Carolina are not publicly available.

We used spatial data from the NC DENR Division of Water Quality to identify the locations of industrial hog operations within 22,860 m of the households that participated in this study. These spatial data have not been updated since 2003. We did not include hog operations that were no longer permitted as of 2015 in our analyses, and the number of new hog operations since 2003 (not included in the NC DENR spatial data) is likely negligible due to a state-wide moratorium on new hog waste lagoon construction since 1995. Still, the location of barns and sprayfields and the number or type of animals on some operations may have changed. Thus, the spatial data we used to model households' environmental exposures to industrial hog operations may not reflect the true distribution of hogs and industrial hog operations in the state. No evidence exists to indicate that misclassification was differential with respect to the exposure or to the outcome.

We did not ask household members to report symptoms of recent skin and soft tissue infection (potentially caused by *S. aureus*) at the time of household environmental sample collection. Previous work has observed a higher prevalence of *S. aureus* infection among individuals who live in rural areas with a high density of industrial livestock operations, including those who do not report direct contact with livestock (Casey, Curriero, et al., 2013). Other studies have linked household reservoirs of antibiotic-resistant *S. aureus* to recurrent skin and soft tissue infections among household members (Davis, Iverson, et al., 2012; Fritz et al., 2014), although the directionality of this exchange is often unclear (Milstone, 2014). Our study design and limited sample size were not appropriate to a) assess prevalence of symptoms of skin and soft tissue infection among household members, or to b) examine whether contamination of the household environment may be associated with such symptoms, if present. Future studies should examine the role of the household environment in these relationships.

Despite its limitations, this study is the first to identify associations between households' environmental exposure to industrial hog operations and percentage of household surfaces contaminated with MDRSA and *S. aureus* CC398. Future studies of household contamination with antibiotic-resistant *S. aureus* in regions with a high density of industrial hog operations should be conducted at a larger scale, employ a more refined spatial exposure model, and should include surface and nasal samples from multiple time points. Improved spatial modeling could reveal additional or stronger associations between households' exposure to industrial hog operations and *S. aureus*-related outcomes in the household environment, including at air deposition sites. A larger sample size could allow us to examine important effect modifiers of these associations (e.g. whether or not pets are allowed on furniture). Repeated sampling of the household environment and simultaneous nasal swab collection from household members could establish temporality and directionality of the relationship between *S. aureus* in the household environment and household members' noses. Overall, our findings add to the growing literature about worker and community exposures to antibiotic-resistant bacteria disseminating from the industrial hog production environment.

TABLES

Table 5.1. Characteristics of 26 households participating in a household environmental sampling study in North Carolina, 2014.[a]

	N[b] (%)
Total number of participating households	26
Individuals living in household	
<3	3 (12)
3-5	19 (74)
≥6	4 (15)
Number of workers living in household	
1	16 (62)
≥2	10 (38)
Occupations of other household members	
Industrial livestock operation	4 (15)
Meat processing plant	1 (4)
Medical facility or health clinic	1 (4)
Any pets inside home	7 (27)
Pets allowed on furniture	4 (15)
Distance to nearest industrial hog operation	
<1000 m	10 (38)
1000-2000 m	6 (26)
≥1000 m	6 (26)
Live on same property as an industrial hog operation	3 (12)
Animals raised on property at home	2 (7)
Hogs	2 (7)
Chickens	1 (4)
Cows	1 (4)
Household's source of health insurance[c]	
No health insurance	16 (62)
Company health insurance	10 (38)
Private health insurance	2 (7)
Public health insurance (e.g., Medicaid)	4 (15)
Place where household members seek medical care[c]	
Private doctor	16 (62)
Emergency department	5 (19)
Free clinic	4 (15)
Hospital	4 (15)
Urgent care center	2 (7)
Company doctor	1 (4)
Do not use any medical care	2 (7)
Household member used antibiotics in past three months	1 (4)
Household member visited hospital or other medical facility in past three months	4 (15)
Household member was admitted to hospital in past three months	1 (4)

[a]All household members in participating households identified as Hispanic.
[b]Totals for each characteristic may not sum to the total number of households due to missing information.
[c]Totals do not sum to 100% because participants could report more than one of the categories.

Table 5.2. Distribution of households and household environmental samples positive for *S. aureus* and related outcomes in North Carolina, 2014.

	Households positive for outcome				Mean % of samples positive for outcome per household					
	At least one site		Air deposition site		All sample types[a]		Touch samples[b]		Face/nose samples[c]	
Outcome	N=26	%	N=23[d]	%	Mean (%)	Range (%)	Mean (%)	Range (%)	Mean (%)	Range (%)
S. aureus	23	88	9	39	47	0, 100	35	0, 100	66	0, 100
MRSA	3	12	2	7	3	0, 43	0	0, 0	5	0, 100
MDRSA	11	42	1	4	11	0, 75	8	0, 60	17	0, 100
scn-negative *S. aureus*	12	46	3	13	15	0, 86	12	0, 75	21	0, 100
tetracycline-resistant *S. aureus*	14	54	2	7	12	0, 56	11	0, 67	15	0, 100
S. aureus CC398	7	27	1	4	6	0, 43	2	0, 33	9	0, 100
S. aureus CC9	4	15	0	--	4	0, 43	5	0, 75	4	0, 100

[a]Comprising touch samples, face/nose samples, and an air deposition site sample (if collected).

[b]Examples of touch samples include refrigerator handles, TV remotes, microwave handles, and kitchen faucets, among others.

[c]Examples of face/nose samples include personal pillows, living room sofas, and living room sofa pillows.

[d]Air deposition site samples were not collected from three households.

FIGURES

Figure 5.1. Proposed role of the household environment in transmission of livestock-associated *S. aureus* among industrial hog operations, people who work at industrial hog operations, and household members in regions with a high density of industrial hog operations.

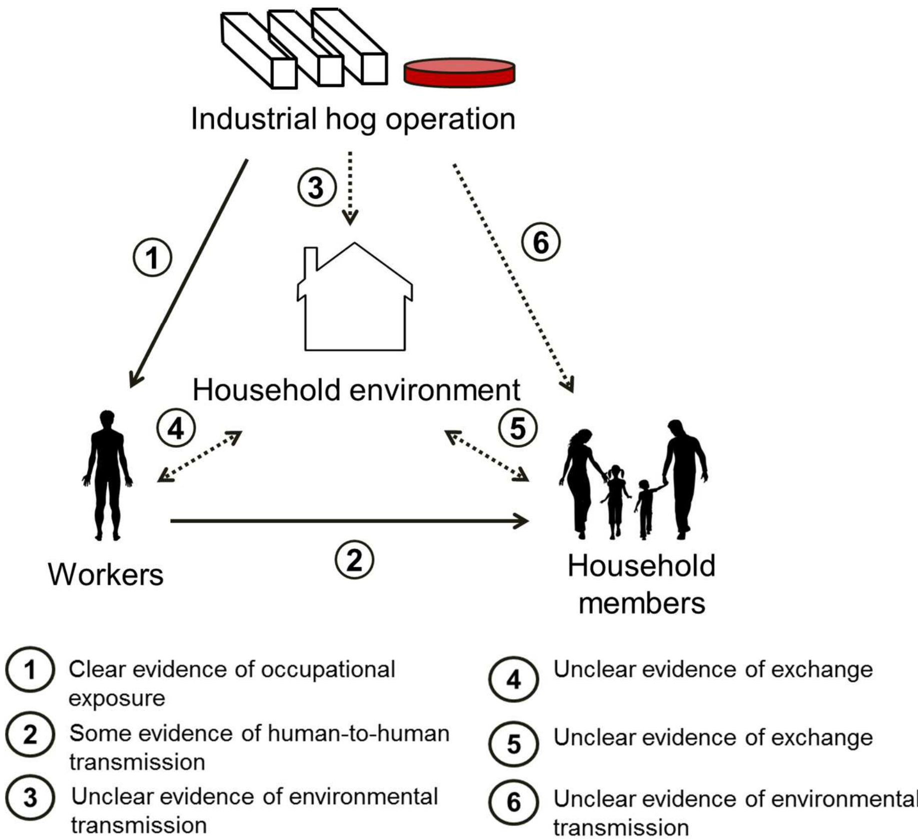
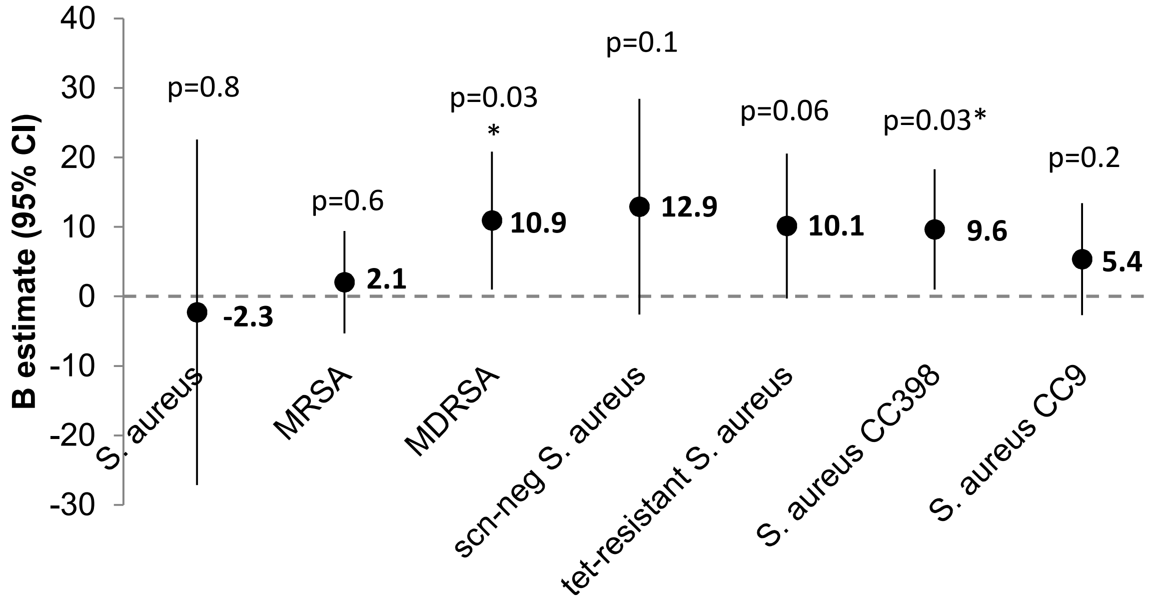


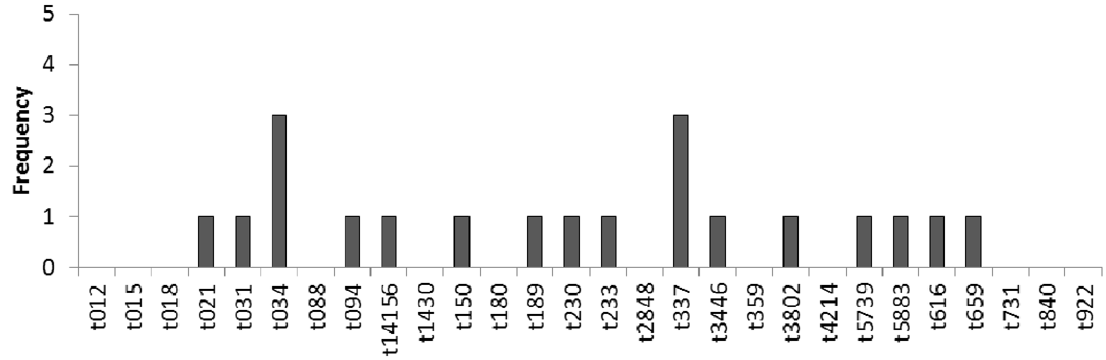
Figure 5.2. Associations between increases in the interquartile range of households' environmental exposure to industrial hog operations and the percentage of household environmental surfaces positive for *S. aureus* and related outcomes.[a,b]



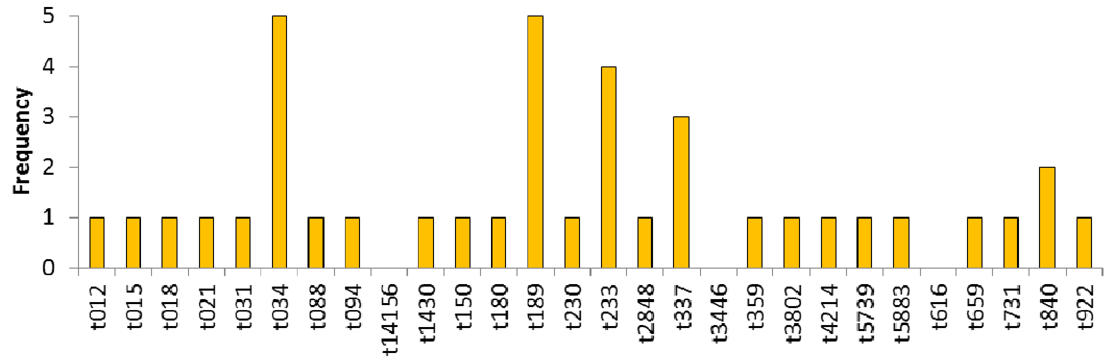
[a]Interquartile range = 22.9 environmental exposure units.
[b]Four households did not provide their household addresses and were thus excluded from analysis.

Figure 5.3. Overlap of *S. aureus spa* types between *S. aureus* isolates recovered from the household environment and *S. aureus* isolates recovered from household members' noses ≤2 weeks prior to household sampling.

a) *S. aureus* spa types detected in the household environment



b) *S. aureus* spa types detected in the noses of household members ≤2 weeks prior to household sampling



c) Number of households in which *S. aureus* spa types were concordant between the household environment and the noses of household members ≤2 weeks prior to household sampling

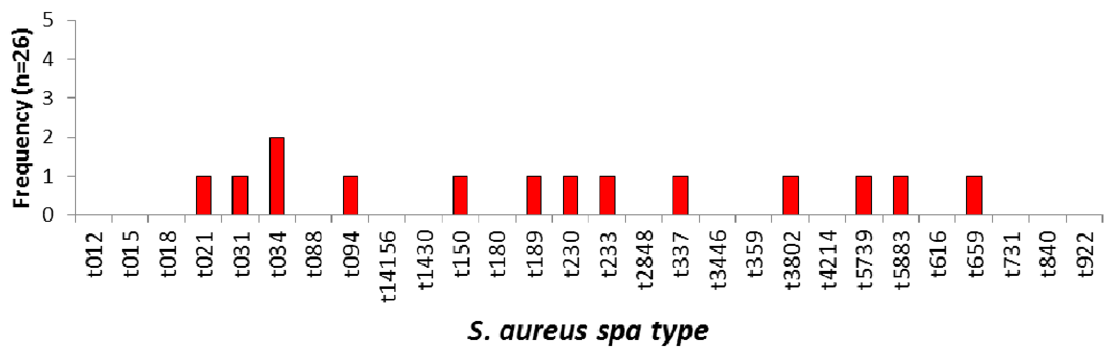
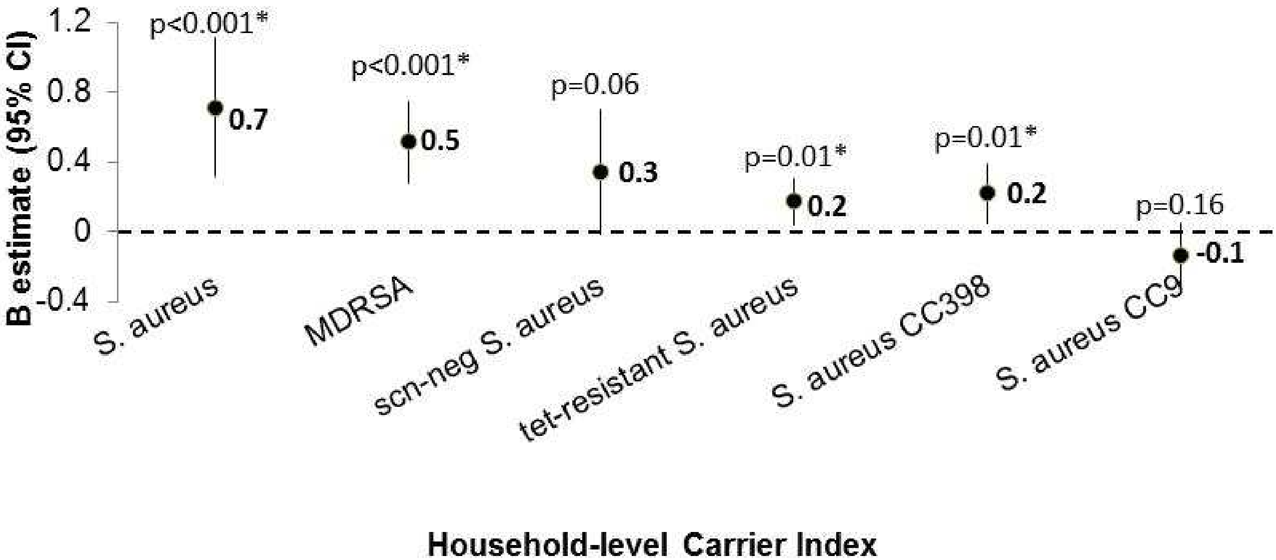


Figure 5.4. Unadjusted associations between household-level carrier indices for *S. aureus* and related outcomes and percent of household environmental surfaces positive for each outcome.



[a]Nasal swab data for ≤2 weeks prior to household environmental sampling was not available for 7/26 households. These households were excluded from analysis.
[b]MRSA carrier indices are not presented because no study participants in any of the 19/26 households carried MRSA ≤2 weeks prior to household environmental sampling.
[b]Each household-level carrier index was calculated by determining the percentage of nasal swabs positive for *S. aureus* or related outcome out of the total number of nasal swabs contributed by household members ≤2 weeks prior to household sampling.

CHAPTER SIX: CONCLUSION

Industrial food animal production operations produce the majority of meat raised in the United States, and their share of domestic pig, poultry, and beef production continues to grow (Walker et al., 2005). Certain CAFO production practices, including the large-scale use of antibiotics and the disposal of animal waste on proximal fields, may negatively impact human and environmental health (Donham et al., 2007; Walker et al., 2005). In 2005, the first reports of a hog reservoir of antibiotic-resistant *S. aureus* in Europe suggested that hog operation workers may be exposed to a novel microbial health risk (Wulf & Voss, 2008).

Studies seeking to investigate the occurrence, persistence, and potential health consequences of livestock-associated *S. aureus* among humans in contact with hogs have been limited in the United States. Hog production in the United States is concentrated in Iowa and eastern North Carolina. Livestock workers in these regions are often a difficult population for academics to engage for surveillance studies. Further, these individuals largely comprise minorities and immigrants, who may be reluctant to participate in academic research studies that require biological samples (Mitloehner & Calvo, 2008; Yancey et al., 2006).

The work outlined in this dissertation, as well as previous research in North Carolina that informed this work (Nadimpalli et al., 2014a; Rinsky et al., 2013), was possible by leveraging existing collaborations between investigators at UNC-Chapel Hill and the Rural Empowerment Association for Community Help (REACH), a community-based organization located in eastern North Carolina. Through ongoing community outreach efforts, REACH has earned the trust of industrial livestock workers and their household members, a population that would otherwise be prohibitively difficult for researchers to engage for surveillance studies. Collaborative efforts by academic researchers at UNC Chapel Hill, Johns Hopkins University, and community organizers from REACH have provided the foundation for the work presented here.

In Chapter 2, I describe nasal carriage findings from a 14-day, repeated measures pilot study of 22 industrial hog operation workers employed in North Carolina. The purpose of this study was to examine short-term changes in nasal colonization following up to 96 hours away from the hog production environment. Workers provided swabs twice daily, once in the morning and once in the evening, during a two week period that spanned at least 24 hours away from the workplace. This study was the first in the United States to examine temporal changes in *S. aureus* nasal carriage among individuals frequently exposed to livestock. We found that nearly half (46%) of workers who participated were persistent carriers of *scn*-negative *S. aureus*, despite time away from work. Six of these workers persistently carried MDRSA, and one persistently carried MRSA. This study supports a growing body of evidence that suggests that individuals who are frequently exposed to livestock may not just be contaminated with zoonotic *S. aureus* in the workplace; instead, they may truly be colonized.

We used findings from this pilot study to inform the design of a four month, repeated measures study of *S. aureus* nasal carriage among industrial hog operation workers and their household contacts, described in Chapters 3 and 4. One hundred and three workers and 80 of their household contacts enrolled; 98 workers and 77 household members completed at least 6/8 bi-weekly study visits over the four month period. During the baseline visit of this study, we assessed nasal carriage of *S. aureus*, including MRSA and MDRSA, and collected reports of recent symptoms of skin and soft tissue infection. Twelve participants, including six workers and six minors, reported such symptoms. These individuals were more likely to carry *S. aureus* in their noses at baseline; workers who reported recent symptoms were more likely to carry MDRSA and *scn*-negative *S. aureus*. Despite small numbers, this is the first study in the United States to report an association between nasal carriage of antibiotic-resistant *S. aureus* and symptoms of infection among individuals directly and indirectly exposed to livestock, although the directionality and temporality of this association could not be assessed from our cross-sectional analysis. Data from this study also revealed that increased frequency of face mask use at work was associated with a lower prevalence of nasal carriage with antibiotic-resistant *S. aureus*, including *S. aureus* with indicators of livestock association. Thus, this study identifies personal protective measures that workers could take that may prevent nasal colonization in the first place.

This study also revealed notable differences in the temporal dynamics of nasal colonization with zoonotic *S. aureus* among workers and household members in eastern North Carolina, compared to agricultural regions in Europe. First, we observed similar distributions of never, intermittent, and persistent nasal carriage of *scn*-negative, tetracycline-resistant *S. aureus* CC398 between workers and their household contacts. Previous work has rarely identified these bacteria in the noses of household members who do not report contact with livestock (Garcia-Graells et al., 2013; Verkade et al., 2014). Second, we recovered *S. aureus* CC9 at least once from almost 40% of workers in this study, although almost never from household members (3%). This clonal complex has rarely been described outside of Asia. Frequent recovery of CC9 from industrial hog operation workers in this study suggests that reservoirs of livestock-associated *S. aureus* may not be as region-specific as previously thought. Third, exposures that were most strongly associated with household members' nasal carriage of *S. aureus* with indicators of livestock association over the four month period were a) the nasal carriage outcomes of workers living in the household, and b) whether or not workers living in the household wore face masks. These findings indicate that workers may be directly or indirectly sharing zoonotic bacteria with their household contacts. The repeated-measures design of this study allowed us to investigate the dynamics of household-level zoonotic *S. aureus* transmission more thoroughly than any other study previously conducted in the United States.

In Chapter 5, I describe the first investigation in the United States of the role of the household environment in the dissemination of zoonotic *S. aureus*. This study may also be the first in the world to investigate household contamination with zoonotic *S. aureus* in a region with an extremely high density of intensive animal production (regional animal density was not provided in the one previous household sampling study we are aware of, conducted by Garcia-Graells *et al*. in Denmark, Belgium, and the Netherlands in 2013). We recovered antibiotic-resistant *S. aureus* with indicators of livestock association, including MRSA and MDRSA, from multiple environmental surfaces in industrial hog operations workers' households. We detected concordance between *S. aureus* recovered from the household environment and *S. aureus* recovered from household members' noses. Further, we observed that industrial hog operation workers' households with greater potential environmental exposure to industrial hog operations had a higher percentage of surfaces in their home contaminated with multidrug-resistant *S. aureus* and *S. aureus* CC398. To our knowledge, this study is the first to identify the potential role of environmental transport in contamination of the household environment with zoonotic *S. aureus*. Environmental sampling of other households in this region where household members are not employed at food animal production facilities is needed to gain insight into this hypothesis.

There were an estimated 292,000 livestock workers employed in the United States in 2012 (Census of Agriculture, 2007; United States Department of Agriculture, 2010; United States Department of Commerce, 2010). Most farm workers in the United States are foreign born (Mitloehner et al., 2008); in both of the surveillance studies described in this dissertation, 99-100% of participants identified as Hispanic. Worker protections are not highly regulated in the United States, particularly for on-site dust levels and animal handling (Mitloehner et al., 2008). Written warnings and safety information that do exist may not be helpful to workers who are illiterate or cannot read English (Mitloehner et al., 2008). Studies conducted in collaboration with community groups who have earned the trust of CAFO workers, such as outlined in this dissertation, can provide unique insight into exposures to zoonotic bacteria among CAFO workers and their household contacts. Importantly, our findings can be verbally reported back to workers and the larger community, in order to increase awareness of the potential microbial risks associated with occupational and environmental exposure to these operations.

S. aureus infections primarily present as skin and soft tissue infections (Wertheim et al., 2005). Livestock exposure is a potential risk factor for *S. aureus* infections (Benito et al., 2014). However, the percent of *S. aureus* infections that occur in the United States' population that are attributable to livestock exposure is difficult to determine due to limited surveillance of this outcome. Even if surveillance data were available, *S. aureus* infections among livestock workers or their household contacts may not be captured. More than half of households that participated in the four month surveillance study (Chapters 3 and 4) did not have health insurance. Farm workers living in rural areas may not seek medical care for skin infections if symptoms are mild or short-lived (Mobed et al., 1992). Immigration status is also known to affect access to medical resources (Hubbell et al., 1991; Ku & Matani, 2001). In the four month cohort study described here, we were unable to determine how frequently symptoms of infection were representative of true *S. aureus* infection. We were concerned that more intrusive data collection methods (e.g. asking participants to allow community organizers to swab their suspected infection sites) may have impeded participants' willingness to answer questions about symptoms. However, despite limitations, the surveillance studies described in this dissertation provide some of the only information accessible regarding the prevalence of symptoms of skin and soft tissue infection among industrial hog operation workers and their household contacts in North Carolina.

The goal of this body of work is to provide microbiological and epidemiological data that can be used to further our understanding of the occurrence, persistence, and dissemination of antibiotic-resistant *S. aureus* associated with the livestock production environment. The findings described here may be used to strengthen academic and community-level efforts to minimize the environmental and public health impacts of industrial food animal production in the United States.

APPENDIX A: CHAPTER 2 SUPPLEMENTARY MATERIAL

Table A1. Differences in survival of *S. aureus* seeded onto nasal swabs when stored at room temperature (25°C) versus refrigeration (4-8°C) over a 10-day period.

	S. aureus CFU/swab	
	10^4	10^2
Day	p-value[a]	p-value[a]
1	0.1697	0.1790
2	0.8112	1.000
3	0.0549	0.4226
4	0.1442	1.000
5	0.0458*	0.6244
6	0.5254	-[b]
7	0.0119*	-
8	0.0162*	-
9	0.0401*	-
10	0.0054*	-

[a]p-value comparing mean *S. aureus* CFU/swab recovered from swabs stored at room temperature versus refrigeration using unpaired, two-sided student t-test and the Satterthwaite approximation.

[b]p-value cannot be computed because observations are too few or because there is not enough variation among observations within a group.

*Statistically significant difference in survival at the 0.05 level.

Table A2. Antibiotics used for susceptibility testing of *S. aureus* isolates

Antibiotic class	Antibiotic tested
aminoglycosides	gentamicin
β-lactams	ampicillin
	oxacillin
	penicillin
cephalosporins	ceftriaxone
floroquinolones	ciprofloxacin[a]
	gatifloxacin[a]
	levofloxacin[a]
	moxifloxacin[b]
glycopeptides	teicoplanin[a]
	vancomycin[b]
lincosamides	clindamycin
macrolides	erythromycin
oxazolidones	linezolid
rifamycin	rifampin
streptogramins	quinupristin/dalfopristin
sulfonamide/methoprim	sulfamethoxazole/ trimethoprim
tetracycline	tetracycline
	minocycline[b]

[a]Tested at UNC-Chapel Hill only

[b]Tested at Johns Hopkins only

Table A3. Distribution of *spa* types, MLST results, and putative CCs among *S. aureus* isolated from 22 industrial hog operation workers in North Carolina over a 14-day study period, stratified by absence of the *scn* gene.

a) *scn*-negative *S. aureus*

spa type	MLST	CC[a]	N[b]	References
t034	---	398	61	(Price et al., 2012)
t337	---	9	47	(Larsen et al., 2012)
t12116[c]	398	398	28	---
t5883	398	398	14	---
t571	---	398	8	(Price et al., 2012)
t2582	---	398	3	(Alt et al., 2011)
t1430	---	9	5	(Feßler et al., 2011)
t4652	398	398	1	---
t3446	9	9	1	---
t2963	20	20	1	---

b) *scn*-positive *S. aureus*

spa type	MLST	CC[a]	N[b]	References
t008	---	8	15	(Deurenberg & Stobberingh, 2008)
t021	---	30	15	(Deurenberg et al., 2008)
t363	30	30	7	---
t2963	20	20	6	---
t346	---	15	3	(Ko et al., 2008)
t5026	72	8	1	---
t878	779[c]	779	1	---
t8890	9	9	1	---

[a]CC was inferred from spa type based on the existing literature unless a putative CC could not be assigned with a high degree of certainty based on *spa* type and the existing literature alone. If this was the case, MLST was performed and CC assignment was based on MLST results.
[b]N refers to the number of *S. aureus* isolates detected with the given *spa* type.
[c]Novel genotype.

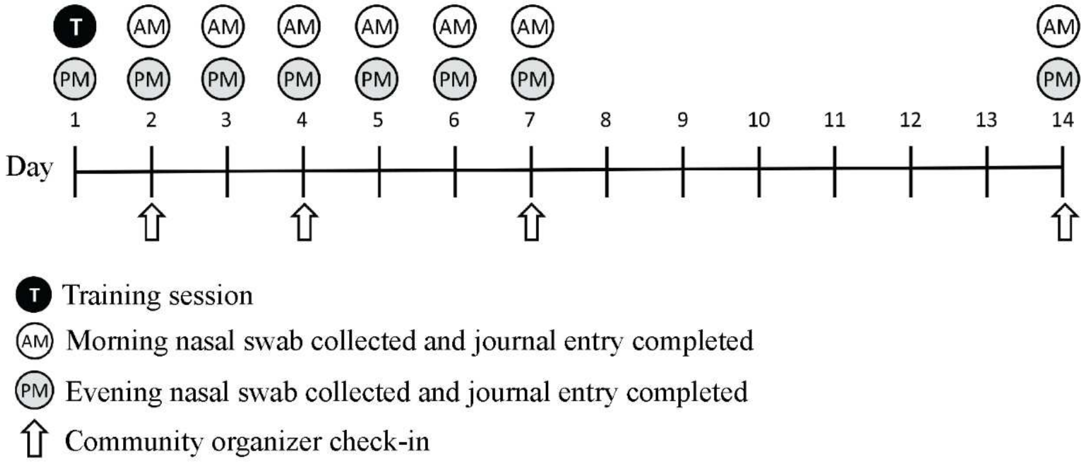
Table A4. Distribution of antibiotic resistance profiles of *S. aureus* isolated from 22 industrial hog operation workers in North Carolina over a 14-day study period.

		Participants		Isolates	
Category	Resistance phenotype	N=22[a,b]	%	N=218	%
Susceptible	--	1	4.5	3	1.4
Single-drug resistant	β-lactams	9	40.9	63	28.9
Double-drug resistant	tetracyclines, β-lactams	4	18.2	38	17.4
	macrolides, β-lactams	1	4.5	3	1.4
	lincosamides, β-lactams	1	4.5	1	0.5
Multidrug resistant	lincosamides, macrolides, β-lactams	3	13.6	10	4.6
	cephalosporins, tetracyclines, β-lactams	1	4.5	5	2.3
	floroquinolones, tetracyclines, β-lactams	1	4.5	1	0.5
	lincosamides, macrolides, tetracyclines, β-lactams	6	27.3	76	34.9
	floroquinolones, macrolides, tetracyclines, β-lactams	1	4.5	1	0.5
	floroquinolones, lincosamides, macrolides, tetracyclines, β-lactams	2	9.1	12	5.5
	aminoglycosides, lincosamides, macrolides, tetracyclines, β-lactams	1	4.5	5	2.3

[a] N refers to the number of participants who carried *S. aureus* demonstrating the specified resistance pattern at least once over the 14-day sampling period.

[b] Numbers do not sum to 22 because some individuals were colonized with *S. aureus* demonstrating more than one antibiotic resistance pattern over the sampling period.

Figure A1. Graphical representation of the 14-day study design and data collection activities.



Time off work occurred between days 1-7 but varied among participants.

Detailed Methods and Results for *S. aureus* survival experiment

Introduction

Due to logistical constraints imposed by the design of this 14-day persistence study, participants' self-collected nasal swabs were stored for up to eight days prior to laboratory analysis. In order to determine whether false negative swabs could result from an eight-day holding time, we conducted a *Staphylococccus aureus* survival experiment prior to beginning the study. In this experiment, we examined the effect of (a) holding times between one to ten days, (b) storage temperature, and (c) initial inoculation concentration on *S. aureus* survival.

Methods

Nasal Swab Seeding

An outline of the study design is provided in **Figure A2**. On Day 0 of the study, we prepared two inoculation solutions with concentrations of 10^5 colony forming units (CFUs)/ml and 10^3 CFU/ml, respectively, using freshly grown *S. aureus* (ATCC 25923) diluted in sterile phosphate buffered saline (PBS). Both solutions were quantified by overnight culture at 37°C on tryptic soy agar (TSA) prior to use. Using sterile conditions, 63 BD BBL™ CultureSwabs™ were inoculated with 100 µl of the 10^5 CFU/ml solution and 63 nasal swabs were inoculated with 100 µl of the 10^3 CFU/ml solution. This resulted in a final concentration of 10^4 CFU/swab among 63 nasal swabs, to mimic concentrations that may be detected among persistent nasal colonizers (Iwase et al., 2010), and a final concentration of 10^2 CFU/swab among the other 63 nasal swabs, to mimic concentrations that may be detected among individuals whose nasal passages are contaminated with *S. aureus*.

Three swabs seeded with 10^4 CFU and three seeded with 10^2 CFU were immediately quantified using procedures described below, in order to obtain baseline counts. For the remaining 120 swabs, half of the swabs seeded with 10^4 CFU (n=30) and half of the swabs seeded with 10^2 CFU (n=30) were stored at room temperature (25°C), in ambient light. The remaining 60 swabs were stored at 4-8°C.

Quantification of *S. aureus* on seeded nasal swabs

We assayed three swabs from each of the four experimental groups (**Figure A2**) on days 1 through 10. Swabs were clipped into 500 µl of PBS and vortexed for 30-60 seconds at high speed. 100 µl of the neat sample and serial 10-fold dilutions there-of were spread on TSA plates using a sterilized spreader and incubated for 24 hours at 37°C. Colonies with *S. aureus* morphology were counted manually or by a destructive counter. The detection limit using this method was 5 *S. aureus* CFU/swab.

Statistical Analyses

We examined the effect of storage time on *S. aureus* survival by constructing time series curves for each initial inoculation concentration. To assess the effect of storage temperature on *S. aureus* survival, we used unpaired, two-sided student t-tests and the Satterthwaite approximation to evaluate the hypothesis that the average *S. aureus* CFU/swab recovered from refrigerated swabs was equivalent to the average *S. aureus* CFU/swab recovered from swabs stored at room temperature (H_o: $\mu_1=\mu_2$) for each day elapsed. We evaluated this hypothesis separately for each initial inoculation concentration. All analyses were conducted using SAS version 9.3 (SAS Institute, Cary, NC).

Results

Ten-day survival curves for *S. aureus* seeded onto nasal swabs at concentrations of 10^4 CFU and 10^2 CFU are presented in **Figure A3**. *S. aureus* was recovered throughout the ten-day period from swabs seeded with 10^4 CFU, regardless of storage temperature. However, *S. aureus* was only consistently recovered (*S. aureus* CFU/swab ≥ detection limit for all three replicates) from swabs seeded with 10^2 CFU through days 1-4.

The effect of storage temperature on survival of *S. aureus* was unclear among swabs inoculated with 10^2 CFU. However, among swabs inoculated with 10^4 CFU, we observed that storage at 4-8°C resulted in greater survival of *S. aureus* compared to storage at room temperature. This effect was statistically significant at p=0.05 on day 5 and after day 7 (**Table A1**).

Conclusions

We conclude that swabs inoculated with 10^4 CFU or higher will reliably be detected by culture following a holding time of up to eight days whether stored at 4-8°C or 25°C. However, swabs inoculated with 10^2 CFU or lower may not be reliably detected by culture after five or more days of storage whether stored at 4-8°C or 25°C. To minimize *S. aureus* die-off before laboratory analysis, we determined that nasal swabs should be stored at 4-8°C following participant self-collection.

Figure A2. Outline of *S. aureus* survival study design.

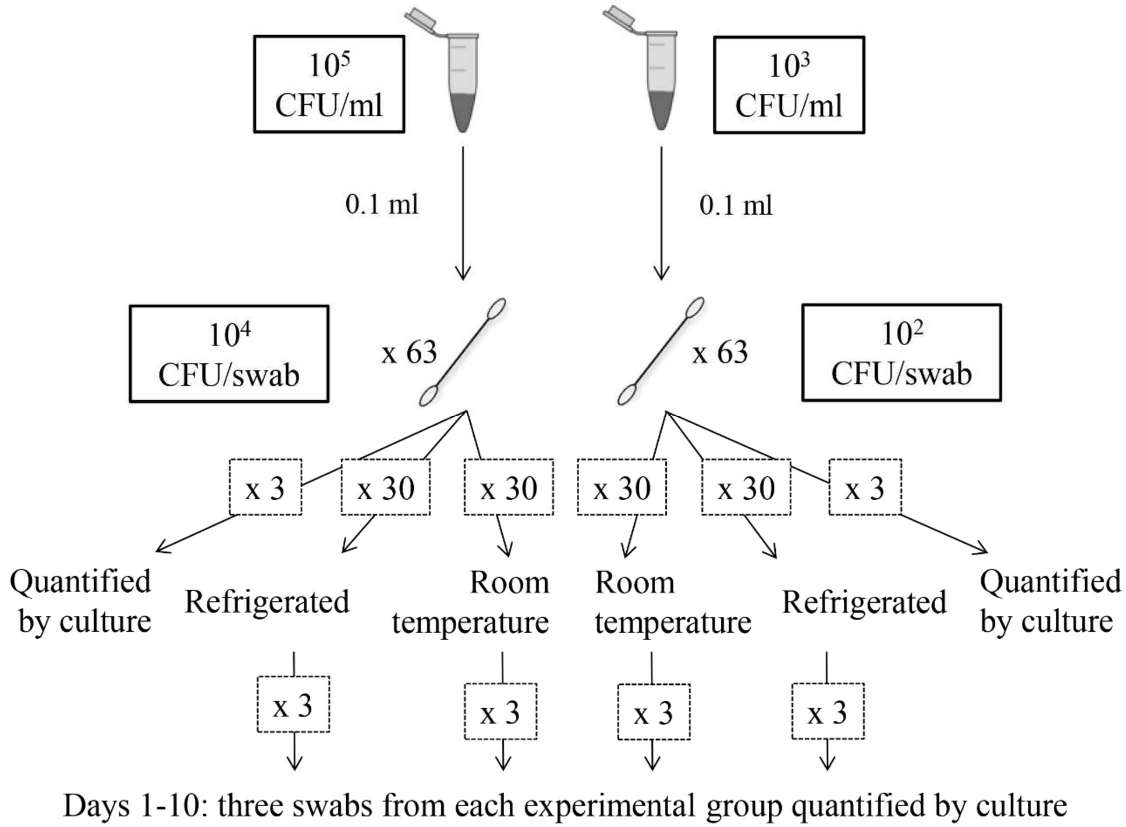
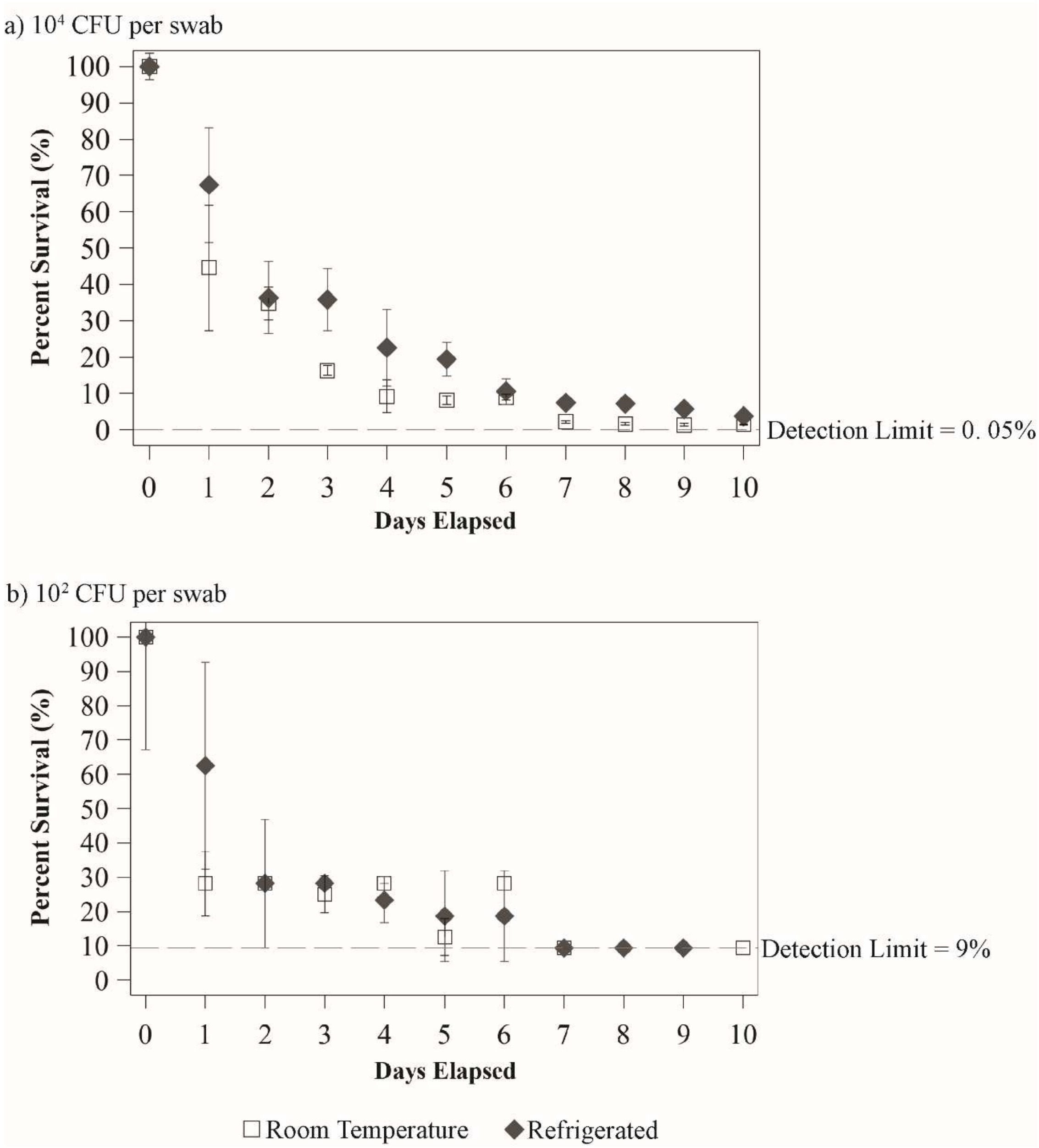


Figure A3. Survival of *S. aureus* seeded onto nasal swabs over a ten-day period.



Markers indicate average *S. aureus* CFU/swab among the three replicates. Error bars indicate standard deviation.

APPENDIX B: CHAPTER 3 SUPPLEMENTARY MATERIAL

Table B1**.** Antibiotics used for susceptibility testing of *S. aureus* isolates.

Antibiotic class	**Antibiotic tested**
aminoglycosides	gentamicin
β-lactams	ampicillin
	oxacillin
	penicillin
cephalosporins	ceftriaxone
floroquinolones	moxifloxacin
glycopeptides	vancomycin
lincosamides	clindamycin
lipopeptides	daptomycin
macrolides	erythromycin
nitrofurans	nitrofurantoin
oxazolidones	linezolid
rifamycin	rifampin
streptogramins	quinupristin/dalfopristin
sulfonamide/methoprim	sulfamethoxazole/ trimethoprim
tetracycline	tetracycline
	minocycline

Table B2. Baseline characteristics of 81 households participating in a cohort study of *S. aureus* nasal carriage in North Carolina, 2013-2014.

	N[a] (%)
Total number of participating households	81
Study participants per household	
1	22 (27)
2	21 (26)
3	33 (41)
4	5 (6)
Individuals living in household	
<3	11 (15)
3-5	53 (73)
≥6	9 (12)
Children <7 years old living in household	33 (45)
Occupations of other household members	
Industrial livestock operation	15 (19)
Pasture-based livestock operation	4 (5)
Meat processing plant	2 (3)
Wastewater treatment plant	2 (3)
Medical facility or health clinic	3 (4)
Any pets inside home	14 (47)
Animals raised on property at home	13 (16)
Hogs	8 (10)
Chickens	3 (4)
Turkeys	1 (1)
Cows	2 (3)
Horses	1 (1)
Household's source of health insurance[b]	
No health insurance	47 (58)
Company health insurance	30 (37)
Private health insurance	8 (10)
Public health insurance (e.g., Medicaid)	11 (14)
Place where household members seek medical care[b]	
Private doctor	44 (54)
Emergency department	18 (22)
Free clinic	15 (19)
Hospital	14 (17)
Urgent care center	11 (14)
Company doctor	1 (1)
Do not use any medical care	4 (5)
Household member admitted to hospital in past 3 months[b]	9 (11)

[a]Totals for each characteristic may not sum to the total number of households due to missing information.

[b]Totals do not sum to 100% because participants could report more than one of the categories.

Table B3. Summary of industrial hog operation worker exposures and symptoms of *S. aureus* infection in past three months in North Carolina, 2013-2014.

	Total N[a] (%)	Symptoms[c] N
Number of participating workers	103 (100)	6
Years employed at current hog operation		
<1	21 (11)	2
1-5	44 (43)	0
6-9	11 (11)	2
≥10	27 (26)	2
Average hours/week		
≤40	19 (19)	0
41-50	31 (31)	3
51-60	40 (40)	3
>60	9 (9)	0
Average number of hogs in contact with per day		
≤1000	64 (67)	4
1001-5000	22 (23)	2
>5000	10 (10)	0
Life stage of hogs in contact with at work[b]		
Sows/farrow piglets/wean/nursery	70 (68)	5
Feeder/finish	29 (28)	1
Direct contact with animals other than hogs at work	8 (8)	1
Work with breeding pigs	24 (23)	4
Draw or collects blood from hogs	9 (9)	1
Give hogs shots	70 (70)	6
Handle dead hogs	79 (79)	5
Eat at hog operation	89 (89)	6
Use of face mask at work		
Always	37 (37)	0
Sometimes	45 (45)	4
Never	18 (18)	2
Use of other personal protective equipment at work		
Always wears gloves	86 (86)	5
Always wears long sleeves and pants, or coveralls	86 (86)	4
Always wears boots or other foot protection	95 (96)	6

[a]Totals for each characteristic may not sum to the total number of workers due to missing information.

[b]Totals do not sum to 100% because participants could report more than one of the categories.

[c]Comprises individuals who reported “Yes, in the past three months” to any of the following: *S. aureus* infection ; skin boil; pus-filled abscess; red, painful, swollen skin bump or “pimple”; or spider bite that is itchy. Participants were shown pictures of *S. aureus* infections with each of these presentations prior to answering this question.

Table B4. Antibiotic resistance patterns of *S. aureus* recovered from the noses of industrial hog operation workers and household members in North Carolina, 2013-2014.

Antibiotic classes for which complete resistance was observed[a,b]	Total no. of classes	Number of participants with resistance pattern	
		Workers N=103 (%)	Household Members N=80 (%)
β-lactams	1	17 (17)	17 (21)
Floroquinolones	1	1 (1)	0
β-lactams, aminoglycosides	2	0	1 (1)
β-lactams, tetracyclines	2	4 (4)	2 (3)
β-lactams, lincosamides, macrolides	3	6 (6)	5 (6)
β-lactams, cephalosporins, tetracyclines	3	1 (1)	0
β-lactams, aminoglycosides, lincosamides, macrolides	4	1 (1)	0
β-lactams, floroquinonlones, lincosamides, macrolides	4	1 (1)	0
β-lactams, tetracyclines, lincosamides, macrolides	4	8 (8)	3 (4)
β-lactams, tetracyclines, lincosamides, macrolides, aminoglycosides	5	2 (2)	0
β-lactams, tetracyclines, lincosamides, macrolides, streptogramins	5	2 (2)	0

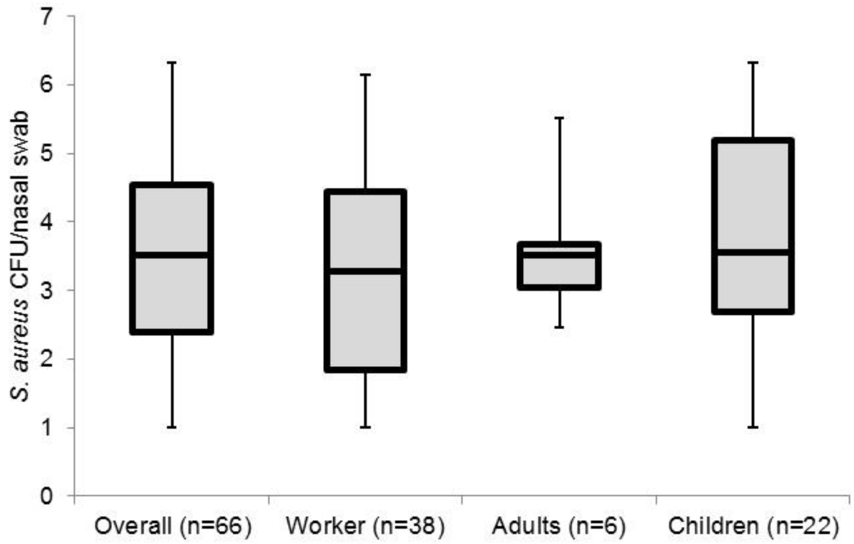
[a]Isolates resistant to three or more class of antibiotics were considered MDRSA.
[b]A list of antibiotics tested is presented by class in Appendix B: Table B1.

Table B5. Distribution of *S. aureus spa* types recovered from the noses of industrial hog operation workers and household members in North Carolina, 2013-2014.

spa type	Overall[a] N=75 (%)	Workers N=45 (%)	Adults N=5 (%)	Minors[a] N=25 (%)
t002	1 (1)	1 (2)	0	0
t008	3 (4)	3 (7)	0	0
t018	1 (1)	1 (2)	0	0
t021	1 (1)	0	0	1 (4)
t031	2 (3)	1 (2)	0	1 (4)
t034[b]	5 (7)	2 (4)	2 (40)	1
t065	4 (5)	3 (7)	0	1 (4)
t089	1 (1)	0	0	1 (4)
t091	3 (4)	1 (2)	0	2 (8)
t094	2 (3)	2 (4)	0	0
t10119	1 (1)	0	0	1 (4)
t1430[c]	1 (1)	1 (2)	0	0
t1446[b]	1 (1)	1 (2)	0	0
t148	2 (3)	0	0	2 (8)
t150	2 (3)	2 (4)	0	0
t189	2 (3)	1 (2)	0	1 (4)
t230[b]	1 (1)	0	0	1 (4)
t233	4 (5)	2 (4)	0	2 (8)
t2868	1 (1)	0	0	1 (4)
t3270[c]	1 (1)	1 (2)	0	0
t337[c]	6 (8)	6 (13)	0	0
t3446[c]	1 (1)	1 (2)	0	0
t346	1 (1)	0	0	1 (4)
t3802	1 (1)	0	1 (20)	0
t4976	3 (4)	1 (2)	0	2 (8)
t571[b]	2 (3)	2 (4)	0	0
t5739	2 (3)	0	0	2 (8)
t5883[b]	1 (1)	1 (2)	0	0
t616	2 (3)	2 (4)	0	0
t622	1 (1)	1 (2)	0	0
t6228	1 (1)	1 (2)	0	0
t645	2 (3)	1 (2)	0	1 (4)
t659	2 (3)	1 (2)	1 (20)	0
t688	1 (1)	0	0	1 (4)
t692	3 (4)	3 (7)	0	0
t701	3 (4)	2 (4)	1 (20)	0
t7226	4 (5)	1 (2)	0	3 (12)

[a]Totals are one less than previously presented as one *S. aureus* isolate from a minor is currently undergoing genotyping.
[b]Associated with *S. aureus* CC398.
[b]Associated with *S. aureus* CC9.

Figure B1. Distribution of *S. aureus* colony forming units (CFUs) among *S. aureus*-positive nasal swabs collected from 183 industrial hog farm workers and their household contacts[a]



[a]Ns do not match Ns presented in Table 2 because some participants' nasal swabs were only positive for *S. aureus* following enrichment. Thus, the number of participants whose nasal swabs we were able to recover quantifiable *S. aureus* from is less than the number of participants.

APPENDIX C: CHAPTER 4 SUPPLEMENTARY MATERIAL

Table C.1. *S. aureus* nasal carriage patterns among 98 industrial hog operation workers and 77 household contacts cultured bi-weekly over four months in North Carolina, 2013-2014.[a]

	Overall	Workers	Household Members	PR[b]	chi-square[c]
	N=177 (%)	N=98 (%)	N=79 (%)	(95% CI)	value (p-value)
S. aureus					
Non-carrier	38 (22)	15 (15)	23 (30)	0.5 (0.3, 0.8)	6.93 (0.03)
Intermittent	80 (46)	51 (52)	29 (38)	1.4 (1.0, 2.0)	
Persistent	57 (33)	32 (33)	25 (32)	0.9 (0.7 1.3)	
MRSA					
Non-carrier	167 (95)	93 (95)	74 (96)	1.0 (0.2, 4.7)	
Intermittent	8 (4)	4 (4)	3 (4)	1.0 (0.9, 1.1)	
Persistent	1 (1)	1 (1)	0	--	
MDRSA					
Non-carrier	110 (63)	54 (55)	56 (73)	0.8 (0.6, 1.0)	6.46 (0.03)
Intermittent	47 (27)	31 (32)	16 (21)	1.5 (0.9, 2.8)	
Persistent	18 (10)	13 (13)	5 (6)	2.0 (0.8, 4.8)	
scn-negative *S. aureus*					
Non-carrier	104 (59)	45 (46)	59 (77)	0.6 (0.5, 0.7)	20.46 (<.0001)
Intermittent	54 (31)	40 (41)	14 (18)	2.3 (1.4, 3.8)	
Persistent	17 (10)	13 (13)	4 (5)	2.4 (1.0, 5.4)	
tetracycline-resistant *S. aureus*					
Non-carrier	118 (67)	54 (55)	64 (83)	0.7 (0.6, 0.8)	15.74 (0.0004)
Intermittent	45 (26)	35 (36)	10 (13)	2.8 (1.5, 5.2)	
Persistent	12 (7)	9 (9)	3 (4)	2.4 (0.7, 9.0)	
S. aureus CC398					
Non-carrier	149 (85)	82 (84)	67 (87)	1.0 (0.9, 1.1)	1.11 (0.57)
Intermittent	18 (10)	12 (12)	6 (8)	1.6 (0.7, 3.9)	
Persistent	8 (5)	4 (4)	4 (5)	0.8 (0.2, 3.3)	
S. aureus CC9					
Non-carrier	135 (77)	60 (61)	75 (97)	0.6 (0.5, 0.7)	
Intermittent	34 (19)	32 (33)	2 (3)	12.4 (3.2, 48.9)	
Persistent	6 (3)	6 (6)	0	--	

scn-negative *S. aureus* CC398					
Non-carrier	155 (88)	83 (85)	71 (92)	0.9 (0.8, 1.0)	3.18 (0.20)
Intermittent	14 (8)	11 (11)	3 (4)	2.9 (0.8, 10.7)	
Persistent	7 (4)	4 (4)	3 (4)	1.1 (0.2, 4.8)	
scn-negative *S. aureus* CC9					
Non-carrier	138 (79)	63 (64)	75 (97)	0.7 (0.6, 0.8)	
Intermittent	32 (18)	30 (31)	2 (3)	12.1 (2.9, 49.4)	
Persistent	5 (3)	5 (5)	0	--	
tetracycline-resistant *S. aureus* CC398					
Non-carrier	154 (88)	84 (86)	70 (91)	0.9 (0.9, 1.0)	1.75 (0.42)
Intermittent	14 (8)	10 (10)	4 (5)	1.9 (0.7, 5.1)	
Persistent	7 (4)	4 (4)	3 (4)	1.1 (0.2, 4.8)	
tetracycline-resistant *S. aureus* CC9					
Non-carrier	149 (85)	73 (74)	76 (99)	0.8 (0.7, 0.8)	
Intermittent	25 (14)	24 (24)	1 (1)	20.5 (2.8, 148.5)	
Persistent	1 (1)	1 (1)	0	--	
scn-negative, tetracycline-resistant *S. aureus* CC398					
Non-carrier	155 (89)	84 (86)	71 (92)	0.9 (0.8, 1.0)	2.54 (0.28)
Intermittent	13 (7)	10 (10)	3 (4)	2.6 (0.7, 9.4)	
Persistent	9 (4)	4 (4)	3 (4)	1.1 (0.2, 4.8)	
scn-negative, tetracycline-resistant *S. aureus* CC9					
Non-carrier	151 (86)	75 (77)	76 (99)	0.8 (0.7, 0.9)	
Intermittent	23 (13)	22 (22)	1 (1)	19.1 (2.6, 144.1)	
Persistent	1 (1)	1 (1)	0	--	

[a]Individuals with no swabs positive for an outcome were defined as non-carriers of that outcome. Individuals with >0% and <80% of swabs positive for an outcome were intermittent carriers. Individuals with ≥80% of swabs positive for an outcome were persistent carriers.
[b]Unadjusted prevalence ratios were generated using log binomial regression models using a generalized estimating equation with an exchangeable correlation matrix to account for the non-independence of observations within households. Household members were the referent group. Stable measurements could not be produced for some associations due to small numbers.

[c]The Rao-Scott chi-square test was used to examine the hypothesis that the distribution of non-carriers, intermittent carriers, and persistent carriers did not differ between industrial hog operation workers and household contacts for each outcome, while adjusting for within-household clustering. Degrees of freedom=2 for all tests. Some tests could not be computed because at least one cell had a frequency of 0.

Table C.2. Concordant *S. aureus spa* types between industrial hog operation workers and their household contacts at the time of bi-weekly nasal sampling during a four month study in North Carolina, 2013-2014.

S. aureus spa type	Characteristics of livestock association: Workers' *S. aureus* isolates				Household members' *S. aureus* isolates				Number of households	Frequency
	scn-negative	tetracycline-resistant	CC398	CC9	*scn*-negative	tetracycline-resistant	CC398	CC9		
t645									1	8
t233									2	6
t233		x							1	1
t189									1	5
t1937									2	3
t701									3	3
t091									1	1
t091						x			1	1
t148									1	2
t150									1	2
t4976									1	1
t4976		x							1	1
t7226									1	2
t008									1	1
t659									1	1
t091		x				x			1	1
t14157	x	x	x		x	x	x		1	1
t189	x				x				1	1
t230			x				x		1	1
t3446	x			x	x			x	1	1
t701		x				x			1	1

APPENDIX D: CHAPTER 5 SUPPLEMENTARY MATERIAL

Table D.1. Characteristics of *S. aureus*, MRSA, and MDRSA isolates recovered from household environmental samples in North Carolina, 2014.

ID	*S. aureus*, MRSA, or MDRSA	*spa* type	Location	Characteristics of livestock association			
				scn-negative	tet-resistant	CC398	CC9
A	MDRSA	t021	TV remote, worker's pillow				
	MDRSA	t021	Refrigerator handles, top of refrigerator	x			
	MDRSA	unknown	Living room sofa				
	MDRSA	unknown	Child's pillow	x			
B	MDRSA	t4214	Living room sofa		x		
	S. aureus	t4214	Kitchen faucets, child's pillow				
C	*S. aureus*	t189	Refrigerator handles	x	x		
	S. aureus	t233	TV remote, living room sofa				
	S. aureus	t233	Worker's pillow	x			
	MDRSA	t233	Child's pillow				
D	*S. aureus*	t189	Worker and child's pillows, top of TV stand, TV remote, microwave handle, refrigerator handles, living room sofa				
E	*S. aureus*	t5739	Refrigerator handle, child's pillow				
	S. aureus	unknown	Worker's pillow				
F	*S. aureus*	t359	Workers' TV remotes	x	x		
	S. aureus	t359	Living room sofa				
G	MDRSA	t034	TV remote	x	x	x	
	MDRSA	t337	Living room sofa	x	x		x
H	MDRSA	t034	Worker and child's pillows, living room sofa	x	x	x	
	MDRSA	t012	Child's pillow				
I	*S. aureus*	t189	Worker and child's pillows, top of refrigerator, TV remote, microwave handle, living room sofa				
	MDRSA	t337	Refrigerator handles	x	x		x
J	*S. aureus*	t233	Workers' pillows, TV remote				
K	MDRSA	t659	Worker's pillow				
	S. aureus	t659	Adult's pillow				
	S. aureus	t840	Refrigerator handle		x		
L	*S. aureus*	t015	TV remote, kitchen faucet				

	MRSA	t034	Living room sofa			x	
	S. aureus	t2848	Living room sofa				
M	MDRSA	t034	Worker’s pillow	x	x	x	
	S. aureus	t233	Child’s pillow				
N	MDRSA	t034	Child’s pillow	x	x	x	
O	*S. aureus*	t150	Worker’s pillow, living room sofa				
P	MRSA	t5883	Living room sofa, back of TV stand	x	x	x	
	S. aureus	t337	Refrigerator handle, TV remote, kitchen faucet	x	x		x
Q	*S. aureus*	t189	Refrigerator handle, TV remote, worker’s pillow and adult’s pillows, top of refrigerator	x	x		
	S. aureus	t189	Child’s pillow	x			
	S. aureus	t233	Living room sofa, child’s pillow				
R	*S. aureus*	t031	Worker, adult, and child’s pillows				
	S. aureus	t922	Living room sofa		x		
	MDRSA	t018	Living room sofa				
	S. aureus	t840	Stove vent hood				
S	*S. aureus*	t731	Child’s pillow				
T	*S. aureus*	t094	TV remote, microwave handle, living room sofa, top of refrigerator, worker, adult, and child’s pillows				
	S. aureus	t094	Kitchen faucet		x		
	S. aureus	t230	Refrigerator handle			x	
U	*S. aureus*	t3802	TV remote, refrigerator handle, living room sofa, top of refrigerator, worker and child’s pillow				
	MDRSA	t337	Kitchen faucet	x	x		x
V	*S. aureus*	t189	Worker and adult’s pillow	x			

Table D.2. Parameter estimates of household-level risk factors as predictors of percent of household environmental samples positive for *S. aureus* and related outcomes, North Carolina, 2014.[a]

	Percent of household environmental samples positive for outcome				
	S. aureus	MDRSA	*scn*-negative *S. aureus*	tetracycline-resistant *S. aureus*	*S. aureus* CC398
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
No. of household members	2.4 (-6.9, 11.7)	0.6 (-5.0, 6.2)	-2.6 (-9.3, 4.1)	-0.7 (-5.5, 4.0)	-2.7 (-5.9, 0.6)
No. of workers	-8.1 (-26.5, 10.3)	0.0 (-11.2, 11.2)	4.0 (-9.5, 17.5)	3.1 (-6.4, 12.6)	6.0 (-0.4, 12.4)
Cumulative hours worked per week(/10) by workers	-1.6 (-5.4, 2.1)	-0.1 (-1.2, 1.0)	0.0 (-2.7, 2.7)	0.5 (-1.4, 2.4)	0.8 (-0.5, 2.1)
Household member visited hospital or other medical facility in past three months	-24.6 (-57.0, 7.8)	0.2 (-14.8, 15.3)	-10.7 (35.1, 13.6)	-4.8 (-22.2, 12.5)	0.34 (-12.4, 13.0)
Pets in house	5.3 (-19.5, 30.1)	3.7 (-11.2, 18.6)	2.8 (-15.3, 20.9)	-4.2 (-16.8, 8.5)	4.1 (-4.9, 13.0)
Pets allowed on furniture	2.2 (-30.6, 35.0)	18.6 (0.3, 36.9)	10.8 (-12.7, 34.3)	17.6 (2.2, 33.0)	14.7 (4.1, 25.2)

[a]All estimates were generated using unadjusted univariate linear regression models. Distributions of household-level risk factors are provided in Table 5.1.

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